October 2001 • NREL/MP-510-31293

Biofuels Program Semiannual Report

Second Half FY2001
April 1 through September 30

R. Wooley

National Renewable Energy Laboratory

1617 Cole Boulevard
Golden, Colorado 80401-3393

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TABLE OF CONTENTS

ABBREVIATIONS AND ACRONYMS

ACS	American Chemical Society
AFUF	Alternative Fuels User Facility
ANOVA	analysis of variance
AOP	annual operating plan
ASTM	American Society for Testing and Materials
ATCC	American-type culture collection
B/MAP	Biomass Agri-Products
BCA	bicinchoninic acid
BDC	Biodiesel Development Corporation
BMG	Bateman Merrick Group
CAFI	Consortium on Applied Fundamentals and Innovation
CCM	cellulase cost matrix
CD	catalytic domain
CIFAR	California Institute for Foods and Agricultural Research
CRADA	cooperative research and development agreement
CSL	corn steep liquor
DACS	Data Acquisition and Control System
DDRD	Director's Discretionary Research and Development Fund
DNA	deoxyribonucleic acid
DOE	Department of Energy
DSA	diafiltration saccharification
DSA	diafiltration saccharification assay
FPRP	Fats and Protein Research Foundation
FTIR	Fourier transform infrared
FTLB	Field Test Laboratory Building
GC	gas chromatograph
GCI	Genencor International
GIS	geographic information system
HBCU	Historically Black Colleges and Universities
HHF	hybrid hydrolysis and fermentation
HPLC	high-performance liquid chromatograph
HTS	high throughput screening
LAP	laboratory analytical procedures
LCA	life cycle analysis
MSDS	material safety data sheet
MW	molecular weight
MWD	molecular weight distribution
NBB	National Biodiesel Board
NBC	National Biofuels Center
NEP	near-term ethanol project
NIR	near infrared spectroscopy
NIST	National Institute of Standards and Technology
NREL	National Renewable Energy Laboratory
NYSERDA	New York State Energy Research and Development Authority
OFD	Office of Fuels Development

ORNL	Oak Ridge National Laboratory
PDU	Process Development Unit
PI	principal investigator
PLS	partial least squares
QA/QC	quality assurance/quality control
R&D	research and development
RBEP	Regional Biomass Energy Program
RFP	request for proposal
RS	reducing sugars
SAT	site acceptance testing
SEC	size exclusion chromatography
SHF	sequential hydrolysis and fermentation
SOW	statement of work
SRM	standard reference materials
SSF	simultaneous saccrification and fermentation
TPD	tons per day
USDA	U.S. Department of Agriculture
WRBEP	Western Regional Biomass Energy Program

ENGINEERED FEEDSTOCKS

Summary of Technical Achievements or Results

A random sampling of 18 freshly harvested corn stover materials, mostly from the year 2000 crop and produced in four states, was collected and analyzed for compositional diversity. Glucan plus xylan content was shown to vary over a range of >8% on a dry weight basis among these samples. Based on variability in polysaccharide content alone, the effect of this degree of variability on process economics is to spread the minimum selling price of ethanol over a range of 20 cents per gallon.

Some variability in pretreatment efficiency was also observed among a subset of four of these stover materials. In one case, pretreatment efficiency exceeded the benchmark case parameters. According to the benchmark process model, the observed increase in pretreatment yield for this sample decreases the minimum selling price of ethanol by about 10 cents/gallon.

We conclude that compositional variation can have fairly significant effects on process economics. The goal of introducing corn breeders to the concept that corn stover quality is a characteristic to which they should be devoting some attention is therefore entirely substantiated.

General Technical or Scientific Progress

Engineered Feedstocks Project

Milestone Progress/Completion

Two P milestone reports were completed during the performance period.

P milestone #295 – "Developments in Plant Breeding and Biotechnology," completed 9/30/01.

P milestone #294 – "Interactions with the Corn Seed Industry," completed 9/30/01.

Progress Highlights and Issues

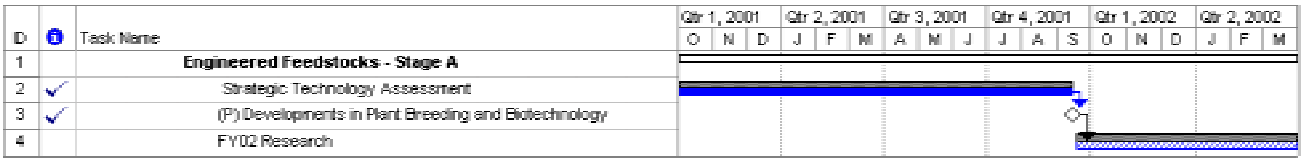

Figure 1: Engineered Feedstocks baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01.

Developments in Plant Biotechnology

The 221st ACS National Meeting in San Diego included a 2-day session on plant cell walls, organized by Drs. Debra Mohnen (Univ. Georgia) and Kanwarpal Dhugga

(Pioneer Hi-Bred International). The program covered topics ranging from basic research on cell wall structure and synthesis to industrial applications of cell wall modifying enzymes. A fair amount of attention is being directed at a series of mutants discovered in Arabidopsis a few years ago that affect the relative ratios of sugars in cell walls. Xyloglucan in this species contains a small amount of fucose (a 5-carbon sugar). In one of these mutants, L-fucose is replaced by L-galactose. Other mutations affect other sugars. This series of mutations, and others that affect cellulose and lignin biosynthesis, make it clear that cell wall composition and architecture are plastic genetic characters. A common conclusion by meeting participants is that it is now possible to contemplate manipulating cellulose quality and content in plant cell walls. Characteristics of cellulose that are targets include: chain length, microfibril size and crystallinity, microfibril angle, cellulose to lignin ratio, and the timing and pattern of cellulose deposition. Additional details can be found in the report for P milestone # 295.

Interactions with Corn Seed Industry

We are in the process of finalizing the analysis of compositional data collected using near-infrared spectroscopy (NIR) from a series of corn stalks provided under Materials Transfer Agreement with Pioneer Hi-Bred International. Data and conclusions from this work are confidential.

Drs. Brad Krohn and Matt Krauss have convinced Monsanto's management team to fund some research in the area of biomass conversion. Of particular interest to the Biofuels Program is the fact that funds were set aside to provide NREL with stover materials from the 2001 crop from 20-30 genetically diverse commercial hybrid varieties grown at up to 6 locations for compositional analysis.

Discussions were held with three other small plant biotechnology companies regarding their interest in diverse areas related to biomass conversion. Two additional requests for using MRI's proprietary intellectual property (E1 endoglucanase), developed using Biofuels funding, have resulted from these discussions.

Genetic and Environmental Effects on Composition of Corn Stover Materials

Milestone Progress/Completion

P milestone #271 – "Genetic and Environmental Components of Compositional Variability in Corn Stover." The milestone report was completed on 4/16/01.

P milestone #272 – "Assessing the Effect of Compositional Variation on Pretreatment Efficiency." Four of the samples from the 2000 corn crop were assessed in duplicate for differential performance in identical batch pretreatment reactions. This milestone report was completed on 7/18/01.

C milestone #273 – "Estimating the Effects of Compositional Variation on Biomass Conversion Process Economics." This milestone was completed on 8/30/01.

Progress Highlights and Issues

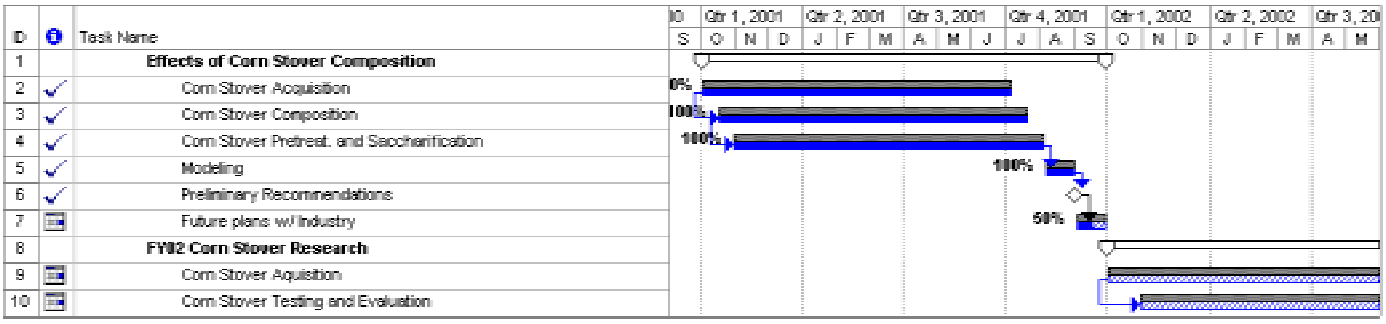

Figure 2: Effect of Corn Stover Composition baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01.

Due to the relatively late inclusion of this project in the annual operating plan (AOP) last fall and the relatively short window available for collecting stover, we could obtain only a small number of genetically distinct stover samples from our contacts around the country. Most of the samples obtained were Pioneer varieties (13 of 18). One sample is a Dekalb variety, and the other four are unidentified hybrids (perhaps mixtures). As Pioneer controls a 40% market share in the U.S. corn seed business, this mix is not as biased as it might have been.

Among the 18 stover samples analyzed, statistically significant differences in NIR-predicted compositions were detectable using Analysis of Variance. These differences correlated with the location where the stover was produced. As we were not able to obtain any of the varieties from more than one location, it is not possible to state whether these differences in composition are the result of genetics or environmental influences, or both. Compositional differences among these stovers were largely confirmed by wet chemical analysis, thus supporting the reliability of the NIR rapid analysis model for raw corn stover. Because they fill a void between samples in the current calibration set for the NIR model for corn stover, the wet chemical analysis from these samples will be incorporated into the calibration set used to update the NIR model for raw corn stover.

Four of the 18 stovers were selected for batch pretreatment experiments designed to detect differences in prehydrolysis performance. Duplicate samples of each stover were subjected to identical pretreatment conditions (165^{o}C, 0.9% H_2SO_4, 8 minutes) in a 1-liter Parr reactor and complete carbon mass balances were calculated for each reaction. Analysis of the data indicates that differences in pretreatment performance may be occurring, but variability of mass balance calculations make this conclusion somewhat equivocal at this time.

A process economic analysis using the benchmark ASPEN+ process model was carried out to estimate the effect of compositional variability on the minimum selling price of ethanol. The glucan plus xylan content of the 18 stover materials studied varied over a range of >8% (dry weight). The effect of this variability in feedstock composition is to spread the minimum selling price of ethanol over a 20-cent/gallon range (i.e., 2.4 cents per gallon ethanol per percent change in glucan plus xylan). Therefore, depending on the

observed differences in polysaccharide content among these 18 samples, the minimum selling price of ethanol varies over a range of 20 cents/gallon ethanol.

A separate economic analysis was carried out to estimate the effect of differential performance in pretreatment on ethanol cost. The pilot plant feedstock material was pretreated to almost exactly the same extent as was observed in earlier Sunds pretreatment runs. Under the conditions employed, one of the feedstock materials gave higher xylan hydrolysis yields than is assumed by the benchmark ASPEN+ model. Therefore, the benchmark ASPEN+ model estimates a decrease in the minimum selling price of ethanol of approximately 10 cents/gallon, relative to the base case.

We conclude that variation in the composition of corn stover, from whatever cause, can affect process economics to a significant extent and at multiple points in the process.

A variety of corn breeders and field trial managers at universities (University of Wisconsin, Iowa State University, University of Nebraska); U.S. Department of Agriculture, Agricultural Research Service (USDA/ARS) (Nebraska, Minnesota, Iowa State); and industry (Pioneer Hi-Bred, Monsanto) have been contacted to arrange access to genetically diverse stover materials from the 2001 crop. We will also be able to obtain a subset of these materials grown in more than one location, in order to assess the interaction between genetics and environment and its effect on stover composition. Arrangements for the acquisition of diverse stover materials from the 2001 crop are being finalized.

Scientific Publications, Presentations, and Other Activities

General Presentations /Travel

- S. Thomas attended the annual meeting of the American Chemical Society (ACS) in San Diego, CA, in April, 2001.

- S. Thomas presented a general talk on biomass conversion at the Fifth Annual Rocky Mountain Plant Biotechnology and Molecular Biology Symposium at Colorado State University, in April, 2001.

- S. Thomas participated in the National Bioenergy Center's Strategic Partnerships Workshop held in Lakewood, CO, in April, 2001.

Scientific Meetings: Papers/Posters Presented or Recently Accepted for Presentation

- D. Templeton presented a poster on compositional variation of corn stover at the ACS National Meeting in San Diego, CA, in April.

- D. Templeton will present a poster on compositional variation of corn stover at the 23rd Symposium on Fuels and Chemicals in Breckenridge, CO, in May.

PRETREATMENT

Summary of Technical Achievements or Results

Good technical progress was achieved in all Pretreatment research and development (R&D) projects during this reporting period. In the Advanced Pretreatment Project, work was focused on the eight Stage A work elements: literature review, hemicellulose fundamentals, pretreatment consortium activities, pretreatment equipment design, new ideas methodology, pilot scale equipment capabilities, enzyme-substrate interactions, and expert panel selection process. Key accomplishments included the successful modification of a two-stage engineering-scale countercurrent pretreatment reactor system to allow for independent operation of the horizontal stage as a high-solids prehydrolysis reactor. This re-configured system has been successfully operated using hardwood sawdust and milled corn stover feedstocks. Other key accomplishments include continued process engineering and other logistical support to the Biomass Refining Consortium on Applied Fundamentals and Innovation (CAFI).

In the Wonderwood (Prehydrolysis Hot Wash) Project, Stage 2 activities were completed and indicated a smaller but still significant potential cost savings using corn stover as compared to previous results using yellow poplar sawdust as the feedstock. Further development of this technology requires development of pilot scale pretreatment capabilities associated with the reconfigured horizontal stage of the engineering-scale pretreatment reactor and the recently ordered Pneumapress filter. Development of these capabilities will be conducted within the Advanced Pretreatment Project. If warranted, further demonstration activities may be conducted within the Enzyme Sugar Platform Project. Additional fundamental studies to determine the long-term potential of the hot washing concept will be conducted within the Advanced Pretreatment and Boundary Layer Projects. As of September 30, 2001, the Wonderwood (Prehydrolysis Hot Wash) Project will be discontinued as an independent project.

A C Milestone for the Boundary Layer Theory Project entitled "Using Fundamental Knowledge Concerning Minimization of Free Energy, Propose Boundary Layer Perturbation Mechanics Necessary for Optimal Reactor Performance and Designs for Aqueous Biomass Fractionation" was completed. It was shown that: 1) the removal of hemicellulosic sugars from a hardwood model feedstock, as measured by xylan removal, is best fitted kinetically by a single first order rate expression and that the "traditional" biphasic model is a result of reassociation of released xylose monomers and polymers; 2) that hardwood lignin solubilization under prehydrolysis conditions can be as high as 50% using flowing reactors and as high as 80% under total hydrolysis conditions and that a fraction of this lignin is primarily monomers and dimers and is a very attractive fraction for upgrading to niche chemicals; 3) that the rate constant for cellulose hydrolysis can be enhanced 50-70X and yields of glucose can be near quantitative by using a shrinking bed reactor as compared to a plug flow reactor; 4) that the intrinsic hydrolysis rate constant for the hydrolysis of the β-1,4 linkage in both xylan and cellulose are very similar, differing only by a factor of 3X, not 150X as is reported in the literature, and 5) that the

preferred conformation of both monomeric glucose and xylose under hydrolysis conditions (>185^{o}C), is the open chain, extremely hydrophilic conformation. This is consistent with the observation that increased temperatures of hydrolysis lead to increased yields of both sugars from biomass but also leads to extreme difficulty in managing the very short residence times required for high yields because it is the open chain conformation that is most vulnerable to undesirable side reactions.

Activities in the Process Development Unit/Data Acquisition and Control System (PDU/DACS) Maintenance Project focused on on-going routine maintenance, deploying an electronic scheduling and tracking database for routine PDU maintenance, and repairing the damaged pretreatment flash tank for the Sunds hydrolyzer.

General Technical or Scientific Progress

Advanced Pretreatment Project

Milestone Progress/Completion

C Milestone #221 -"Complete Modification of Countercurrent Pretreatment Reactor System for Single Stage Operation" (6/30/01). This milestone was successfully completed on time. Numerous components of the original reactor configuration were removed, coupled with the installation of a 130 L Jaygo high solids mixed pressure vessel to serve as the solids collection tank for the reconfigured system. The reconfigured system has been successfully operated in a single stage dilute sulfuric acid prehydrolysis mode using yellow poplar sawdust as the feedstock to validate that the modifications to the system are complete. This system is now fully operational as a pilot scale pretreatment reactor system and is available for utilization by projects that require such a capability. This system may be useful in achieving high solids pretreatment for a corn stover feedstock due to the forced movement of material through the horizontal reactor via its screw conveyor.

C Milestone #237 - "Economic Evaluation of Various Pretreatment Technologies" (6/30/01). This milestone was completed on 9/30/01, representing a 3 month delay from its original due date. The delay was primarily related to delays in securing the services of a contract engineer to perform this work. The process engineering and analysis team reviewed and modeled the two ammonia pretreatment processes with the respective members of the Biomass Refining CAFI. Lime and hot water had been modeled in the first half of the year; the completion of the ammonia process models completes the pretreatment models for the CAFI. To help refine the models with data, we provided a template to each research group for collecting data during experiments. We plan to provide on-going economic analysis support for the group as they continue their work under the USDA grant.

Progress Highlights and Issues

Since the successful completion of the milestone to re-configure the two-stage engineering-scale countercurrent pretreatment reactor to allow for independent operation of the horizontal stage, efforts have been underway to to obtain preliminary pretreatment process performance on corn stover in that reactor.

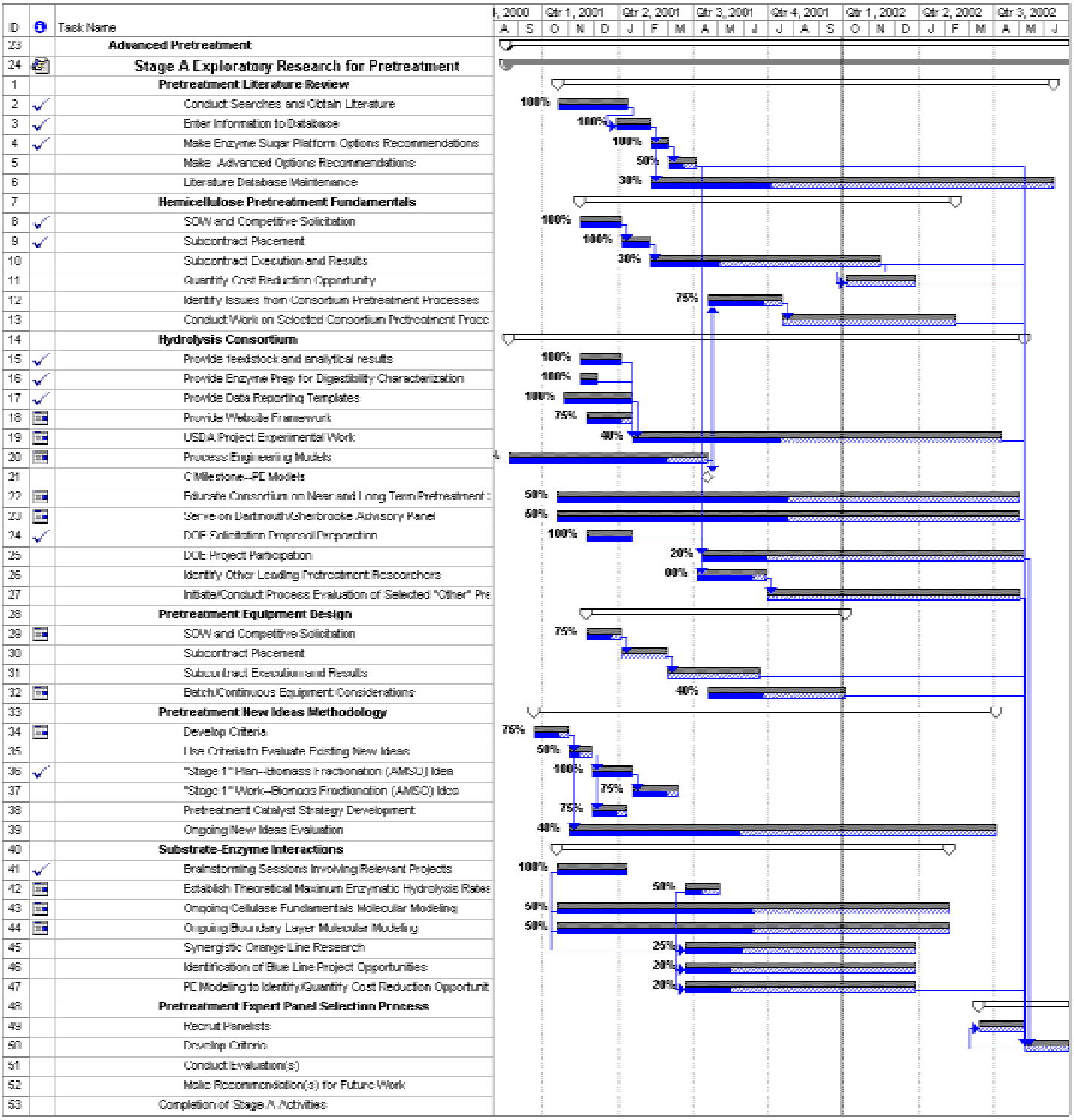

Figure 3: Advanced Pretreatment. Effect of Corn Stover Composition baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01

Two key goals are to determine if high solids loading (above 20%) using corn stover can be achieved and to determine if short residence times (2-4 minutes) are possible in this reactor. Achieving both of these conditions will begin to approach the corn stover

pretreatment conditions in the NREL 4 L batch steam gun that achieved high xylose yields (~90% of theoretical). The initial process performance data will be generated by the Advanced Pretreatment Project. If warranted, further activities using corn stover in this reactor may be conducted under Stage 3 of the Enzyme Sugar Platform Project.

A 3 ft 2 inch pilot scale Pneumapress pressure belt filter has been ordered and is expected to be delivered in January, 2002. This filter will serve many of the general solid-liquid separation needs in the PDU and will provide better process data on solid-liquid separations for use in process engineering models. In addition, this filter is capable of performing the hot separation and washing operations at the pilot scale for the hot washing process developed in the Wonderwood (Prehydrolysis Hot Wash) Project.

The placement of a subcontract to evaluate pretreatment reactor equipment design and materials of construction requirements has been delayed due to a broadening of the initially developed Statement of Work (SOW). This subcontract now seeks to evaluate reactor design and materials of construction considerations for a broad variety of pretreatment technologies (including those being developed by the Biomass Refining CAFI) instead of just further refining the existing dilute acid materials of construction knowledge base. This subcontract will now be placed early in FY 02.

Prototype testing of a new screw design for the vertical stage of the engineering-scale countercurrent pretreatment reactor system has also been delayed. Other on-going work in the Advanced Pretreatment Project and the Boundary Layer Project is providing new insight into reactor design requirements that will impact any re-design of the existing vertical stage.

Subcontractors:
Y.Y. Lee, Auburn University
ADZ-1-31084-01
Investigation of Hemicellulose Hydrolysis Fundamental Kinetics Using Dilute Sulfuric Acid
7/30/01 - 4/30/02
This recently-placed subcontract in focusing in 3 major tasks: 1) establish baseline kinetics of xylan depolymerization and xylose formation from corn stover using dilute sulfuric acid in a batch mode 2) determine and evaluate different model reactor systems on corn stover xylan hydrolysis kinetics and 3) determine oligomer characterization and oligomer conversion kinetics of corn stover prehydrolyzates.

Wonderwood (Prehydrolysis Hot Wash) Project

Milestone Progress/Completion
C Milestone # 307 - Efficacy of a Hot Washing Process for Corn Stover and Potential for Near Term Deployment" (8/31/01). The milestone was successfully completed on August 31, 2001. Pretreated corn stover was produced that had similar characteristics to Wonderwood (yellow poplar sawdust). The cost reduction observed with yellow poplar was again obtained with corn stover, but to a lesser extent. Under a compressed time

schedule to better align this project with the timeline of the Enzyme Sugar Platform Project, the milestone represented the completion of Stage 2 of this project.

Progress Highlights and Issues

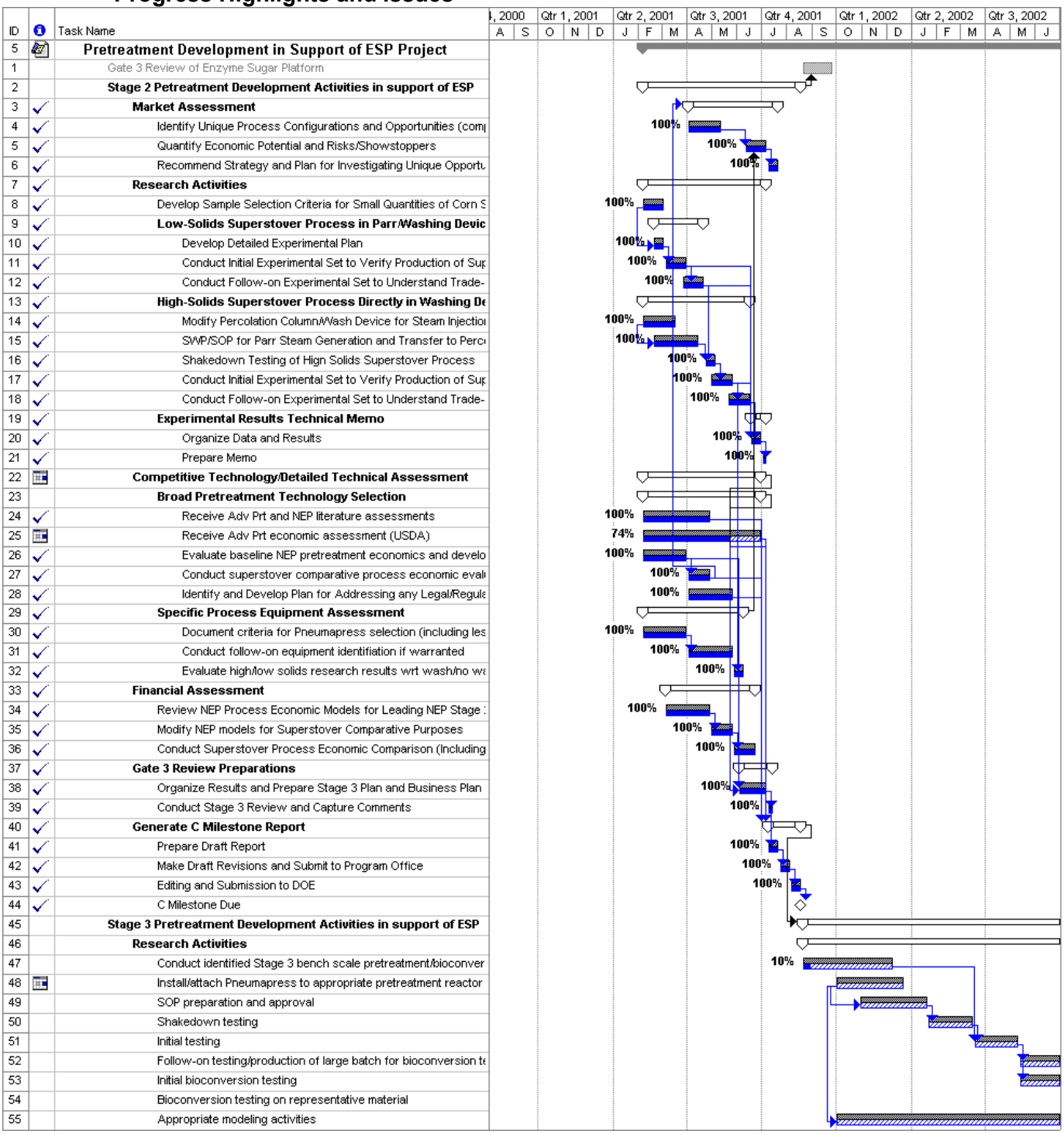

Figure 4: Wonderwood Project. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01

A Gate 3 review for this project was held on August 14, 2001. The results obtained during the Stage 2 work were presented to the Enzyme Sugar Platform Project to determine if this technology meets the criteria for that project's pretreatment technology selection. Potential cost reductions were demonstrated using this technology with corn stover, but to a lesser extent when compared to those achieved with yellow poplar sawdust. The issues resulting from the review indicated that while the preliminary results were positive, they needed to be demonstrated and confirmed at the pilot scale.

Critical issues that were identified in the gate review included developing a better understanding of the fate and effect of the relatively high lignin solubilization that occurs in this process configuration. While the milestone was completed on schedule, both the low and high solid corn stover experimental tasks required greater resources than planned due to unexpected problems resulting from feedstock handling, pretreatment, and analytical issues.

The decision resulting from the gate review was to discontinue the Wonderwood (Prehydrolysis Hot Wash) Project as a distinct project. Initial development of equipment capabilities to demonstrate this process concept at a pilot scale will be conducted within the Advanced Pretreatment Project. If the results of this initial testing meet the requirement of the Enzyme Sugar Platform Project's pretreatment selection criteria, further development and demonstration may be conducted within that project. The study of more fundamental aspects resulting from this project that might lead to further cost improvements will be investigated within the Advanced Pretreatment and Boundary Layer Projects. Finally, an informal technology brief designed to highlight the soluble lignin co-product opportunities will be developed for use by the Industrial Partnerships Team.

Boundary Layer Theory (Hydrolysis/Fractionation Fundamentals) Project

Milestone Progress/Completion

C Milestone # 222 – "Using Fundamental Knowledge Concerning Minimization of Free Energy, Propose Boundary Layer Perturbation Mechanics Necessary for Optimal Reactor Performance and Designs for Aqueous Biomass Fractionation," (09/30/01) was completed as scheduled and submitted. Extensive use of computer modeling of the very dilute-acid and hot compressed water hydrolysis/fractionation of a hardwood model feedstock through subcontracted work at Cornell University and Accelrys, Inc. in concert with additional subcontracted work at Auburn University and in-house bench scale work as well as in-house computer modeling using molecular mechanics, has lead to novel and innovative fundamental knowledge of the free energies required for an effective and economical fractionation of biomass. New insights into the intrinsic fractionation of all three polymeric species in biomass have lead to innovative kinetic models. The use of these models will allow NREL researchers insights into exploring new perturbation techniques at the molecular level and reactor designs to reduce fractionation severity and thus, reduce hydrolysis/fractionation capital and operating costs.

Progress Highlights and Issues

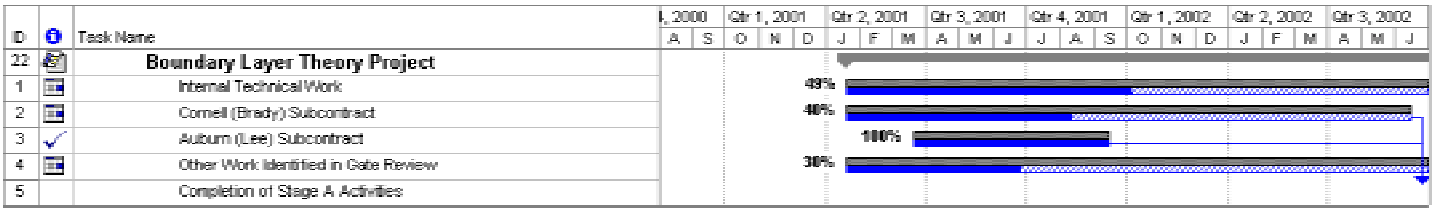

Figure 5: Boundary Layer. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01

Two key achievements were made during this reporting period. The first is the establishment of a very successful three-way consortium of molecular modeling capabilities to study the free energy molecular fundamentals of aqueous hydrolysis/fractionation of biomass. The collaborators are Dr. John Brady at Cornell University, Dr. Keith Glassford of Accelrys, Inc., and Robert Torget of NREL. The modeling packages include both molecular mechanics and quantum mechanics. The goal of the computer-modeling group is to delineate the free energy barriers to successful fractionation of desired products from biomass and propose molecular perturbation mechanisms, which will lead to effective reactor designs. The second achievement is establishment of another very successful team approach to study and propose new global heterogeneous kinetics of the dilute-acid mediated pure cellulose hydrolysis. The members include Par Pettersson, a Ph.D. candidate from Mid-Sweden University, Qian Xiang, a Ph.D. candidate from Auburn University, Dr. Y.Y. Lee of Auburn University, and Robert Torget from NREL. This group will be developing and incorporating mass transfer resistances into cellulose hydrolysis kinetic models as well as developing a comprehensive global model to explain the different hydrolysis rates obtained using pure cellulose in three reactor configurations: batch, fixed volume percolation, and the shrinking bed percolation reactor.

Subcontractors:

John Brady, Cornell University
XDH-0-30009-01
Molecular Modeling of Structured Water Boundary Layer Under Hydrolysis Conditions
4/10/00 – 9/15/01
The subcontractor has conducted simulations that theoretically verify the hypothesis that structured water exists at the surface of cellulose at the high temperatures of dilute acid total hydrolysis and that a significant density barrier exists due to this structuring which would definitely affect free diffusion of released products to the bulk medium. A manuscript has been prepared and submitted to *Biopolymers* entitled "Computer Simulations of Water Structuring Adjacent to (1,0,0) Microcrystalline Cellulose Iβ Surfaces," by C.E. Skopec, P. Zuccato, J.W. Brady, R.W. Torget , and M.E. Himmel.

Y.Y. Lee, Auburn University
ACO-1-31003-01
Investigation of Less Severe Hydrolysis Conditions Using Shrinking Bed Apparatus
02/08/01 – 12/31/01

Work has involved the development of mathematical kinetic models for both hemicellulose and cellulose hydrolysis. We are seeking to understand the heterogeneous kinetic resistances and how these resistances can be addressed by different reactor designs in hopes of designing more effective and less capital intensive hydrolysis reactors.

Keith Glassford, Accelrys, Inc.
Simulating Xylose Ring Opening in the Gas and Liquid Phase
06/01/01 – 09/30/01
Work has involved the quantum mechanical calculations of two conformations of xylose, the ring-chair-equatorial conformation and the open chain conformation at both room temperature and 190^{o}C. The objective is to develop a physical-chemical hypothesis of why increasing hydrolysis temperatures increase yields of both hemicellulosic and cellulosic sugars in a thermochemical process.

Two-Stage Dilute Acid Project

Milestone Completion/Progress

P Milestone # 328 - "Effect of Operating Parameters on Continuous Countercurrent Extraction of Hemicellulosic Sugars from Pretreated Softwood" was completed on May 19, 2001, ahead of schedule but with a reduced scope. This study concluded that good soluble sugar recovery from pretreated wood chips with low water usage was achieved with a countercurrent screw extractor. Key parameters having the most impact on the sugar recovery efficiency are: water temperature, water drainage rate through the pretreated material, and inclined angle of the extractor. However, the pilot unit used is not suitable for handling fine biomass materials, possibly because of inadequate draining area. As a result, no model was developed for the extractor as was originally planned.

Progress Highlights and Issues

The Gate 3 review of the Southeast Alaska Bioenergy Project in January 2001 identified two key Stage-2 tasks that need to be completed before Stage-3 work can begin. These are: (1) feedstock characterization and (2) confirming >50% glucose yield. TSS Consultants (Rancho Cordova, CA) is currently surveying feedstock suppliers for the Ward Cove site. Samples of wood residues from a local veneer mill and surrounding saw mills are being collected for characterization. NREL is carrying out two-stage dilute acid experiments to confirm the glucose yield. Two types of feedstock are being tested: bark-rich hog fuel and sawdust. A funds-in contract was signed with Bateman-Merrick Group (BMG), the Southeast Alaska Project Manager, to provide technical support in planning Stage-3 work. A second Gate-3 review for this project will be conducted in November 2001.

During the performance period, the process engineering team completed a process design package for 2-stage dilute acid technology based on the 1998 Quincy Library Group work. The NREL report, published in September, provides a conceptual design and economic analysis for the process, which does not utilize enzymatic hydrolysis. While similar to the Southeast Alaska project process, this process design varies in that it is for a larger plant (800 TPD) and utilizes continuous hydrolysis reactors.

Process Development Unit/Data Acquisition and Control System (DACS) Maintenance

Milestone Progress/Completion

P Milestone # 333 - "Implementation of a Database Driven System for Managing PDU Maintenance and Operations Tasks" (9/30/01). A web-based system has been developed. PDU safety inspection logs and safety tasks have been entered into the database. A weekly e-mail notification to NREL staff assigned to particular tasks has been implemented, along with a PDU activity scheduling template.

Progress Highlights and Issues

Receipt of parts required for the repair of the agitator of MX-205 (Sunds pretreatment reactor flash tank) from the Netherlands was severely delayed. The parts only recently arrived in the United States and are currently awaiting customs clearance. This has significantly delayed the repair of this system. A hoisting rig and secure platform for the 1600 lb lid of this tank has been fabricated and all disassembly of required components has been completed. Once the replacement parts arrive at NREL, is should take from 4 to 6 weeks to complete the repairs and re-assemble the flash tank.

Subcontractors:

Braconier
TCO-0-30039-01
Process Development Unit Mechanical Services
5/1/00-4/31/02
Major activities included the design and construction of a hoisting rig and secure stand for the lid of the Sunds pretreatment reactor flash tank .

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

- R. Elander and R. Torget attended several meetings of the Biomass Refining CAFI in Breckenridge, CO on May 7-10 2001.

- R. Torget and Par Pettersson attended a training/collaboration meeting with Dr. John Brady concerning molecular mechanics modeling at Cornell University in Ithaca, NY from May 17-20, 2001.

- R. Elander participated as a member of the Panel of Experts for a review of the Dartmouth College/University of Sherbrooke pretreatment fundamentals project in Sherbrooke, Quebec, Canada on June 11, 2001.

- R. Elander gave a presentation on the NREL pretreatment strategy at the Golden Field Office Biomass Review Meeting in Golden, CO on August 16, 2001.

Scientific Meetings: Papers/Posters Presented or Recently Accepted for Presentation

- Torget, R.W.; Kim, J.S.; and Lee, Y.Y. "The Application of Perturbation Mechanics to Delineate the Intrinsic Carbohydrate Fractionation Kinetics in Hardwoods." Oral presentation at the 23rd Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO, May 6-9, 2001.

- Nagle, N.; Elander, R.; Newman M.; Rohrback, R.; Ruiz, R.; and Torget, R. "Efficacy of a Hot Washing Process for Pretreated Yellow Poplar to Enhance Bioethanol Production." Poster presentation at the 23rd Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO, May 6-9, 2001.

- Kim, K. H.; Tucker, M. P.; and Nguyen, Q. A. "Effects of Operating Parameters on Continuous Countercurrent Extraction of Hemicellulosic Sugars from Pretreated Softwood." Poster presentation at the 23rd Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO, May 6-9, 2001.

- Elander, R. "Thermochemical Depolymerization of Biomass Carbohydrates." Accepted for oral presentation at the 5th Biomass Conference of the Americas, Orlando, FL, December 17-21, 2001.

- Elander, R., Nagle, N., and Torget, R. "Examining the Potential for Waste Product Reduction and Energy Saving Techniques in Thermochemical Dilute Sulfuric Acid Processes for Biobased Products and Bioethanol Production." Accepted for oral presentation at the American Institute of Chemical Engineers National Meeting, Reno, NV, November 6-9, 2001.

- Torget, R.W., "Use of Molecular Mechanics, Perturbation Mechanics, and Quantum Mechanics as Tools for Biomass Fractionation Reactor Designs." Accepted for oral presentation at the American Institute of Chemical Engineers National Meeting, Reno, NV, November 6-9, 2001.

Scientific Journals: Papers Accepted for Publication

- Torget, R. "Aqueous Fractionation of Biomass Based on Novel Carbohydrate Hydrolysis Kinetics." US Patent 6,222,419, May 8, 2001.

BIOPROCESS DEVELOPMENT

Summary of Technical Achievements or Results

Corn stover process development activities continued to be the focus of Bioprocess Development researchers during the reporting period. The main emphasis was on pursuing Stage 2 work elements of the Near-term Ethanol Project, particularly techno-economic process modeling, life cycle analysis, pretreatment technology selection, and fermentation strain identification. Secondary emphases were on strengthening our capabilities to analyze corn stover feedstocks and process intermediates with higher accuracy and greater efficiency using rapid near infrared spectroscopy-based methods. Limited experimentation was also carried out to better understand our pretreatment capabilities and to produce representative process residue solids.

Several important accomplishments were achieved during the reporting period:

- We updated the Corn Stover Plan. The new version of this plan is substantially improved over the previous plans and now is consistent with and incorporates the multi-year near-term ethanol project (NEP) corn stover project work (NEP is being renamed the Enzyme Sugar-Ethanol Platform or ESP in FY02). A major accomplishment, which significantly improves the usefulness of the plan, is that the corn stover development aspects of the plan are now based on input from USDA and ORNL. The new plan clearly shows the highly parallel and high risk nature of NEP. The advantage of this parallel approach is that it allows us to greatly compress the project's timeline. The disadvantage is that this reduced timeline means that the risk of not meeting the ultimate project goal is high, since a delay in any one of the project's multiple parallel paths can impact the overall project's timeline.

- We conducted a successful interim Stage 2 "Detailed Investigation" project review to assess progress to date and clarify the specific additional work elements that will be completed prior to conducting a formal Gate 3 project review; successful completion of the Gate 3 project review is proposed as a K-level milestone in the FY02 Annual Operating Plan. We currently anticipate completing the Gate 3 project review within the first half of FY02.

- We continued to make significant progress in terms of outreach.
    - Numerous presentations were made to industry associations containing important project stakeholders (e.g., California Institute for Food and Agricultural Research, International Fuel Ethanol Workshop, Society of Industrial Microbiology).

    - We met with and made presentations to key companies (e.g., Cargill, Novozymes North America) and to essential government collaborators (USDA ARS WRRC, ORNL, DOE GO).

- Building on previous efforts, we strengthened our relationships with the enzyme development and cost reduction subcontractors (Genencor International and Novozymes Biotech).

- We refined previously developed NIR/PLS spectroscopic analysis methods for rapidly determining the composition of raw corn stover and solid corn stover process intermediates and identified limitations in the existing wet chemistry-based methods. New methods have been developed specifically for the compositional analysis of corn stover. Sixteen constituents can now be quantified, providing a more complete compositional profile. These improvements in the calibration methods will improve the accuracy and robustness of the NIR/PLS rapid analysis methods. The NIR/PLS rapid biomass analysis methods are supporting several NREL projects.

General Technical or Scientific Progress

Near-term Ethanol Project

Milestone Progress/Completion

P Milestone # 255 - "Iowa Case Study for Life Cycle Analysis" (5/31/01). The objective of this milestone was changed to submitting a document for peer review in FY02 after updating the modeling results to reflect initial reviewer feedback. Preliminary results of the life cycle analysis of converting corn stover to ethanol were reported in a number of forums where feedback on the initial results was solicited. Based on the feedback, we are conducting additional modeling work using USDA's Century model for soil carbon effects and Oak Ridge's geographic information system (GIS) model for corn stover collection and transportation. The new Century model results were delivered to NREL during the reporting period, and we are now awaiting the revised results of the GIS modeling being done by ORNL. The draft report is being prepared in parallel as we incorporate the Century model data in our updated life cycle model. We plan to complete the data analysis and the preliminary peer review document in early FY02.

P Milestone # 256 - "Compositional and Storage Stability Study: Year 2 Results" (6/30/01). This milestone was completed during the current reporting period, and portions of this work were presented at the ACS Conference in April and the 23rd Symposium on Biotechnology for Fuels and Chemicals in May. The objective of this work is to determine if significant changes to corn stover composition occur during outdoor storage. Two additional rounds of samples collected from the B/MAP corn stover collection facility in Harlan, Iowa, during the past year were analyzed by wet chemical and NIR spectroscopy methods (68 samples have been analyzed using wet chemical analysis methods, 163 samples analyzed by NIR, and 50 samples analyzed by both methods). The samples collected to date span 4 harvest years (1997-2000) and elapsed storage ages of 0-27 months. The analysis of variance (ANOVA) results based on the wet chemistry- and NIR-based methods disagree, however, and this is an important finding. The implications are twofold. First, we cannot draw firm conclusions about what is happening to corn stover composition during long-term storage except to reiterate that compositional changes with potential economic significance are observed. Second, comparing the two methods has highlighted weaknesses in the existing wet chemistry-based methods that need to be resolved to allow us to develop the more accurate and

robust NIR calibrations needed to detect compositional trends occurring with storage time. Additional work to refine compositional analysis methods for corn stover feedstock is planned for FY02.

C Milestone # 224 - “Conduct Interim Stage 2 Review,” (9/28/01) previously “Conduct Gate 3 Review” (4/30/01). The magnitude of the requisite Stage 2 work effort grew during the reporting period as we improved our understanding of the full scope of the project. For example, the collection and evaluation and of technical information on the various options for pretreatment and fermentative microorganisms--in addition to the time needed to get stakeholder buy-in on the technology selection protocols and the development of compelling techno-economic scenarios (including scenarios exploring co-location, co-product, and financial alternatives)are each tasks much larger in size than originally planned. As a consequence of scope expansion, this milestone (which had previously been delayed until 7/31/01) was further delayed to 9/28/01 and its objective/title was modified. The interim Stage 2 review (rather than a Gate 3 review) was successfully completed at the end of the performance period. The interim Stage 2 review documented the good progress that has been made towards completing the Stage 2-level market and financial assessments, selecting key technology elements, and clarifying expectations for outside project support (e.g., by USDA, ORNL, etc.). We also defined what must be completed before we are ready to carry out a Gate 3 review and move into Stage 3. We plan to complete Stage 2 and conduct the Gate 3 project review in the next reporting period.

C Milestone # 225 - “Update Corn Stover Plan With Life Cycle Analysis Input and Colloquy Results” (7/31/01). This milestone was successfully completed on schedule. The new version of the Corn Stover plan is more robust and comprehensive than plans developed in 1999 and 2000. Instead of limiting the focus to corn stover collection, the plan now embodies the multi-year plan for the Enzyme Sugar Platform, in which the corn stover work is subsumed. The corn stover development aspects of the plan are based on input from USDA and ORNL. The ability to involve these two critical organizations in the development of the plan represents a major accomplishment and significantly improves the its value. The new plan reveals the highly parallel nature of the Enzyme Sugar Platform project (formerly known as the Near-term Ethanol Project). The advantage of such a parallel approach is that it allows us to greatly speed up the timeline. The disadvantage is that the highly parallel structure substantially increases the risk of not meeting the ultimate project goal, since a delay in making progress along any one of the major parallel paths can impact the overall project timeline.

C Milestone # 226 - "Complete 4 to 6 Month Objective as Defined in Stage 3 Work Plan" (9/30/01). This milestone was delayed until FY02 due to the delay in the Gate 3 Review (C Milestone # 224 above).

Progress Highlights and Issues

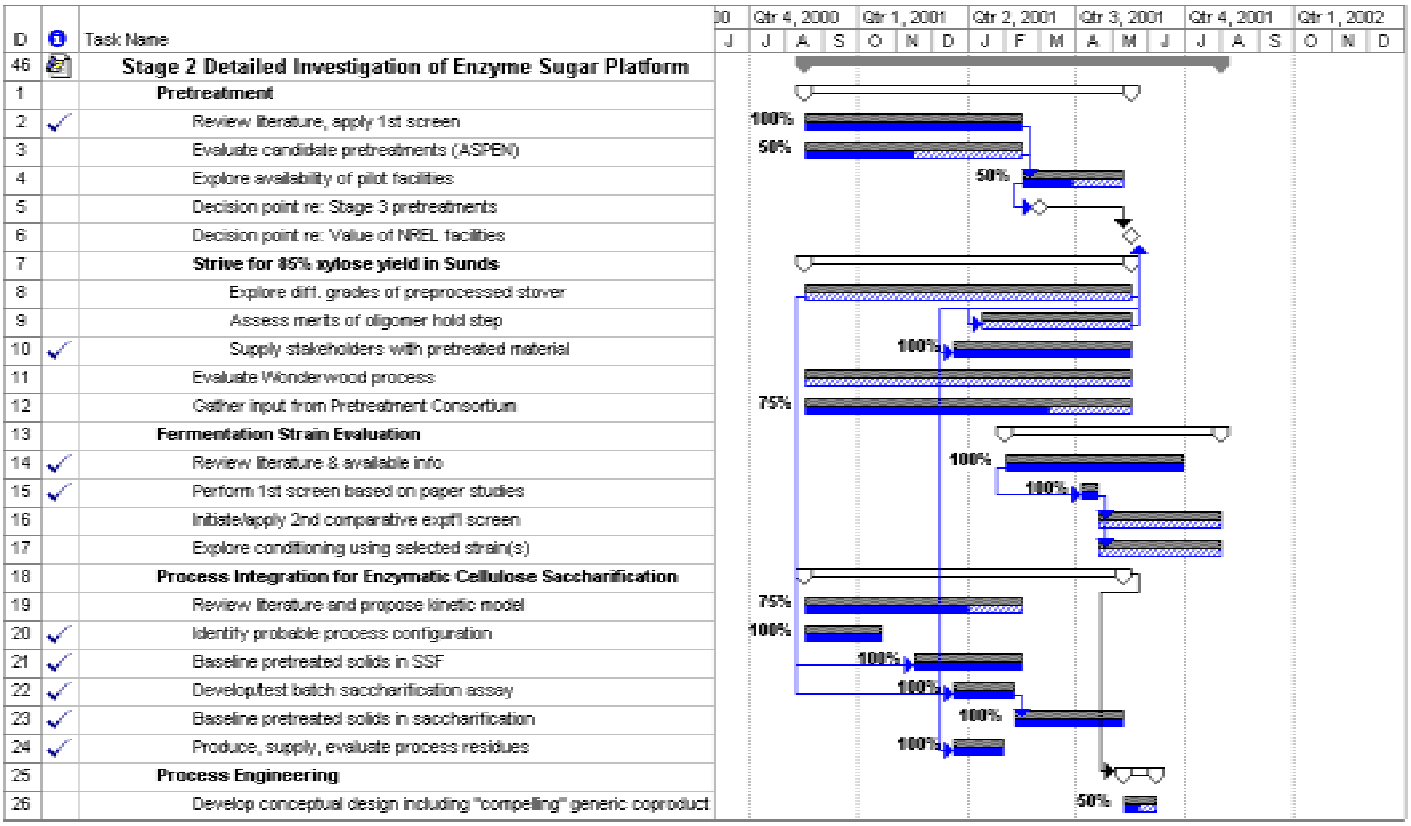

Figure 6: Near-term Ethanol Project Stage 2. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01

The multi-year Near-term Ethanol Project remained in Stage 2 during the reporting period. The focus areas during the reporting period were in the areas of techno-economic process modeling, life cycle analysis, pretreatment technology selection, and fermentation strain identification. Secondary emphases were on strengthening our capabilities for analyzing corn stover feedstocks and process intermediates with higher accuracy and greater efficiency using rapid NIR spectroscopy-based methods; and on defining the enzymatic hydrolysis characteristics of pretreated corn stover in a stand-alone batch hydrolysis mode, i.e., without simultaneous fermentation. Limited experimentation was also carried out to better understand our pretreatment capabilities and to produce representative process residue solids.

The major focus of techno-economic modeling is to develop compelling scenarios and selecting pretreatment and fermentative strain technologies in a manner that engages the respective stakeholders, technology owners, and/or technology champions. This focus is consistent with the input received from the Gate 2 review panel in January 2001.

These Stage 2 efforts are significantly behind our original schedule, however. While substantial progress has been made, the full scope and the difficulty of these efforts was insufficiently recognized in our FY01 planning. The literature reviews and initial

screening based on paper studies are completed for candidate pretreatment processes, but we are still completing process engineering analyses and surveying technology owners to determine the status of options insufficiently reported in the literature. We have also completed the literature review and primary screening of candidate fermentation strains. Comparative secondary screening will not commence until after technology owners have been contacted. We currently expect to complete these remaining technology selection and process engineering tasks within the first half of FY02 and to then commence Stage 3.

The planned experimental pretreatment work has fallen significantly behind plan. Delays in receiving different grades of preprocessed stover, coupled with reactor problems (broken internals requiring substantial repairs), as well as the need to install new high temperature pretreatment processing capabilities were the causes. Consequently, the work to strive for 85% xylose yields and to demonstrate high solids pretreatment, as well as testing of the "wonderwood" concept (now known as "superstover" or "stover hot wash") now will not begin until the second half of FY02.

Better progress according to our original plan has been made in preliminary process integration. We have reviewed the literature and proposed a kinetic model and are currently testing the robustness of this model formulation; model validation will proceed thereafter. After holding additional meetings with the enzyme developers, we have defined the probable cellulose saccharification/biomass sugar fermentation process configuration tentatively to be high temperature stand-alone enzymatic saccharification followed by mesophilic enzymatic saccharification and fermentation. We have developed a batch (stand-alone) enzymatic saccharification assay and baselined the cellulose conversion performance of pretreated corn stover solids in both SSF and using this new stand-alone saccharification assay. We also have produced sufficient quantities of representative process residue solids to enable preliminary evaluation of combustion characteristics and potential coproduct uses and value to be assessed.

The following sections describe progress achieved in each of the major work areas pursued during the reporting period.

Corn Stover Procurement and Compositional Evaluation

Biomass Agri-Products (B/MAP) LLC of Harlan, Iowa, the contractor for the subcontract entitled, "Prepare and Supply Corn Stover for Pretreatment and Bioconversion Testing," made significant progress in procuring and installing equipment to enable dirt to be washed out of field-collected corn stover. Final testing of this equipment is expected to occur early in FY02, whereupon B/MAP will prepare several grades of washed corn stover that exhibit different particle size specifications. These different grades will be tested at NREL to determine which is most suitable for processing in the AFUF's PDU front-end equipment. Thereafter, B/MAP will prepare a large batch of the grade that exhibits the best handling characteristics and will supply it to NREL for pretreatment testing.

The results of the second year of the compositional analysis study were written up, as described previously (P Milestone #256). Overall, the results to date show small but

potentially economically significant changes in composition between harvest years and with storage time. A more thorough examination of the impact of geographic and temporal variation in corn stover composition is being carried out by the Engineered Feedstocks project, Genetic and Environmental Effects on Composition of Corn Stover Materials, and we plan to heavily leverage their results in FY02. Preliminary results from the Engineered Feedstocks project suggest that large compositional differences exist in corn stover produced in different locations (see P Milestone # 272). We also plan to assess the importance of bale mass loss ("shrinkage") on composition by incorporating shrinkage study results that we expect to be obtained in FY02 through an ORNL-funded subcontract.

Corn Stover Pretreatment

See Figure 6 for details of pretreatment activities originally planned for Stage 2. A key objective is to identify the pretreatment technology to be further developed and demonstrated during Stage 3 of this project. Two efforts support this goal. The first is a Stage 2 paper study surveying the performance, development, and demonstration status of potentially available pretreatment technologies. During the reporting period, we evaluated the public literature and began soliciting information directly from technology developers. Members of the NEP and Pretreatment Research teams reviewed the public information and entered it into a Microsoft Access database that will provide us with an effective analysis tool as well as a permanent record for this element of the NEP project as well as for other Pretreatment Research team projects. The initial screening steps involved in identifying promising pretreatment technologies have now been completed. Once information is obtained from the technology developers and the process engineering is completed for the leading candidate approaches, the NEP and Pretreatment teams will work jointly to recommend the best pretreatment option(s) to pursue in the Stage 3 technology development work phase of the project.

The second effort is to experimentally evaluate the capabilities of dilute sulfuric acid pretreatment of corn stover using the NREL PDU's 1 ton/day continuous pilot scale Sunds Hydrolyzer pretreatment reactor. Pursuing this work is essential to benchmark the performance of the dilute acid pretreatment technology using pilot scale equipment and determine if previously unrecognized issues are present. We previously identified acceptable baseline pretreatment conditions and produced initial batches of representative corn stover solids for the subcontracted enzyme developers to use in their enzyme improvement and performance testing work.

During the reporting period, we explored our ability to achieve further improvements in pretreatment performance. Unfortunately, corrosion damage to the agitator system in the exhaust "flash" tank halted this work relatively early on and the bulk of the performance period has been spent disassembling this vessel and agitation system and waiting for new parts to arrive (from Europe). We did determine, however, that it is difficult to process material at input stover solids loadings above about 25% w/w and plan to see if higher solids loading pretreatment is capable using the smaller 200 kg/day continuous engineering scale Sunds reactor in FY02 (see C Milestone # 307). We also plan to try to incorporate the "wonderwood" or "superstover" concept into our baseline pretreatment in

FY02, provided that dilute acid pretreatment remains the front runner candidate following the Gate 3 review.

Support of Enzyme Cost Reduction Subcontract Liaison

We continued to engage the enzyme developers in discussions and to work with researchers at NREL involved in the Enzyme Subcontract Liaison Project to improve the quality and relevance of the metrics and cellulase performance assays being used to benchmark and measure improvements in cellulose hydrolysis efficiency. The project leader met with representatives of each of the enzyme developers to discuss cultivation of hyper-producing fungal strains on pretreated corn stover.

Residue Production

Numerous external stakeholders are interested in evaluating the characteristics and potential value of the lignin-rich solid residues that remain after corn stover has been pretreated and enzymatically saccharified. External stakeholders who have expressed interest in obtaining samples of corn stover process residues include USDA (researchers at various ARS sites across the country are investigating the potential co-product value of solid process residue as a source of valuable sterols, as a substrate for biomaterials, and as a soil amendment); Colorado State University (researchers are exploring residue biosorption characteristics); University of Colorado (researchers are exploring the sedimentation and filtration characteristics of the residue); Cargill Dow; Biofuels Lignin Fuels and Lignin Combustion Projects Biomass Power projects and an OFD business development consultant interested in show and tell samples for discussions on co-firing bioethanol residues in coal-fired power plants. In addition, NREL researchers need residue materials to define the residue's solid-liquid separation characteristics and to further improve the robustness of the sample set used to calibrate the rapid spectroscopic compositional analysis method used for corn stover process intermediates.

During the reporting period, a larger-scale residue production run was carried out in order to produce sufficient material for additional stakeholder evaluation and to support internal research needs. This residue production run used pretreated corn stover that had been produced in the PDU's Sunds pretreatment reactor during the preceding reporting period and also used the general simultaneous saccharification and fermentation (SSF) methodology previously established at smaller scale in the preceding reporting period.

Briefly, the SSF run was conducted using one of the PDU's 1450-L fermentors with a cellulase enzyme loading of 25 FPU/g cellulose.(*S. cerevisiae* D5A was the fermentation microorganism, and was supplemented with 1% w/w corn steep liquor to provide essential nutrients.) This run was completed in late July 2001 and produced a total of approximately 30 kg (dry basis) of washed solid lignin-rich residue. Once compositional analysis is performed and an material safety data sheet (MSDS) for shipping out the material is completed, appropriately sized portions of the residue material will be distributed for evaluation to external and internal researchers.

Life Cycle Analysis of Corn Stover-to-Ethanol

We are finding that our efforts to engage stakeholders in the design and review of the life cycle analysis (LCA) of corn stover for ethanol production offers a tremendous

opportunity for outreach and buy-in about the implications of utilizing corn stover to make biofuels. As a result of stakeholder input, we have been pushed to include important but complex issues such as soil health and sustainability in our analysis. The preliminary results of the life cycle study have been presented to a variety of audiences as part of our effort to broaden stakeholder input and buy-in. As a result, we are now expanding our modeling efforts.

Our preliminary findings suggest that:

1. Carbon sequestration can be increased dramatically by switching from corn-soybean rotations to continuous corn rotations, while replacing traditional intensive tillage practices with low or no till practices.

2. Collecting corn stover to make ethanol eliminates most of the sequestration that can be achieved by switching to low or no till practices in a continuous corn rotation.

3. Despite the reduced sequestration of carbon, overall avoidance of CO_2 emissions is about 50% greater when stover is collected and used to make ethanol, compared with the non-collection scenario.

Being able to show the environmental and economic sustainability of corn stover collection for biofuels production is essential for the success of this project. As such, we plan to expand the current LCA during FY02 to consider multiple scenarios of crop rotation and tillage using the USDA Century model to see how these affect the amount of corn stover available and the sustainability of its use for producing fuels and chemicals. In addition, we also plan to increase the sophistication of the LCA modeling by:

1. Integrating soil erosion modeling directly into the soil carbon model

2. Evaluating stover collection effects on soil carbon and soil health using different soil carbon models (i.e., besides USDA's Century model)

3. Modeling the effects of a corn stover use on water quality

4. Incorporating the impacts of nitrogen fertilizer

5. Integrating corn grain and corn stover collection and use to develop a broader view of the sustainability of ethanol from corn

6. Introduce energy crops as an optional crop rotation

7. Include states other than Iowa to increase the relevance of the study to the nation as a whole

Process Engineering and Technology Selection

A key objective of Stage 2 is to better define the process technology and to justify that its attributes are sufficiently attractive to warrant further development in Stage 3. Part of this

analysis is to explore the economics and economic sensitivities of the process in greater detail and to develop specific performance targets for the Stage 3 development effort. Narrowing the selection of key technology elements, e.g., pretreatment process and fermentation strains, are also important components of the Stage 2 process refinement task.

Significant progress was made during the reporting period to update the ASPEN Plus-based process engineering model that defines how a particular set of feedstock and operation parameters translates into a projected minimum ethanol selling price. Specifically, a revised reference case process engineering model was developed that incorporates the "super stover" or "hot wash" improved pretreatment concept and assumes a hybrid enzymatic hydrolysis and fermentation scheme. This "Process Case" includes the ultimate research targets being aimed for in this project. Key sensitivities were run using the updated process model. We also developed a baseline "Current Economics Case" model that incorporates parameters that represent the current status of technology (what we've proven to date or what we believe current costs to be, e.g., for enzymes). We have documented the rationale behind why we are confident that we can improve the technology from the "Current Economics Case" to the "Process Case" for each of the critical processing steps where improvements need to be achieved.

In addition to improvements to the "Process Case" and "Current Economics Case," as a prelude to a more rigorous second screen on the candidate pretreatment technologies (see pretreatment technology selection below), we completed process engineering models of several dilute acid catalyzed, pH-neutral (hot water), and base (ammonia) catalyzed candidate pretreatment technologies. Variations in reactor configurations for the front-runner dilute acid pretreatment were also evaluated, for both continuous and batch (steam gun) options, as well as a continuous option based on the "super stover" concept. Results to date continue to point to some variation of dilute acid pretreatment as the most viable option.

For profit companies will only be interested in getting involved in and partnering with this project (i.e., participating in Stage 3 and leading the Stage 4 and beyond work effort) if the economic return is sufficiently attractive and the process concept is perceived to represent a real market opportunity. Thus, developing processing scenarios that exhibit compelling economics based on credible and defensible market and performance assumptions is essential for project success. The effort to develop such a scenario constituted a major portion of the process engineering work carried out during the performance period. Scenarios under development include those based on research stretch targets, those based on co-location opportunities, those based on potential co-products, and those based on assuming different financing options. We have developed a preliminary spreadsheet matrix that contains the scenarios run to date that enables us to more readily examine realistic combinations of multiple scenarios (i.e., enabling reduced capital investment, operating cost, lower cost financing, etc.) that may become possible as the project moves forward into Stage 3 and we identify a commercialization partner.

Pretreatment Technology Selection

An important Stage 2 objective is to identify which pretreatment technologies out of the many that have been reported should be considered for inclusion in the Stage 3 "development" phase of the NEP project. To this end, a paper study was initiated during the reporting period to survey the publicly available literature and private sector experts in order to assess the status (i.e., demonstrated efficacy and readiness) of potentially available pretreatment technologies. This effort, which includes soliciting information directly from pretreatment technology developers (since many have not published extensively on their systems), is still underway and is expected to continue into early FY02.

The first phase of the survey consisted of reviewing the existing pretreatment technology literature and was completed towards the end of the reporting period. To capture the results of the literature review in a useful manner, members of both the NEP and Pretreatment Research teams entered key information from the most informative published reports on the various technologies into a Microsoft Access database. To be able to rank order the pretreatments and identify those with the best potential for meeting the NEP's performance requirements and schedule constraints, a set of sequential screening criteria was developed. These sequential screens are being applied to evaluate and compare and ultimately identify the select few pretreatment technologies that will be explored in greater detail in Stage 3.

The first screen asks whether or not there are literature reports showing the pretreatment to exhibit effective performance, which we defined to be at least 75% hemicellulose yield and 80% enzymatic cellulose digestibility, or an equivalent total sugar yield. The second screen seeks to confirm quantitative performance abilities but also asks more qualitative questions relevant to the third level of screening to determine if the candidate pretreatment can meet NEP's demonstration and deployment objectives within its tight schedule requirements. In addition to asking for verifiable information on pretreatment performance (i.e., hemicellulose or xylose yields, enzymatic cellulose digestibility, ethanol yields, etc., that are supported by rigorous carbon mass balances, the second screen asks if sufficient performance information is available to enable an economic evaluation to be performed or verified (since it's critical that we be able to show via simulation that the technology is potentially economically viable before it can be considered for Stage 3 experimental development).

While applying the first screen quickly excluded some pretreatments, there were insufficient data available in the literature to be able to screen out many types of pretreatment. In such cases, for example for pretreatments being investigated as part of the Biomass Refining (Pretreatment) CAFI, as well as for several other industry-based pretreatments, the first screen was simply bypassed since we didn't want to prematurely reject potentially effective pretreatments for which good information was simply not available in the open literature.

Based on the available literature, six pretreatments passed the first criteria: dilute sulfuric acid (as either a batch or continuous process, alone or in combination with a hot wash process), sulfur dioxide, dilute nitric acid, hot water, ammonia percolation, and peroxide

percolation. Some of these pretreatments are also champion-based technologies, meaning that researchers or companies outside the NREL Bioethanol Project are actively championing their development and in many cases are being funded by DOE and/or USDA. The complete list of pretreatments to be evaluated using a second screen are shown in the table below.

Pretreatment Technologies Carried Over into the Second Level of Screening

Pretreatments Selected for Second Screen	Reactor Configuration	Contact/Technology champion
Dilute Sulfuric Acid	Continuous Batch Hot Wash *Pressure Gravity Reactor	NREL NREL NREL /Genahol
Sulfur Dioxide	Continuous	
*Dilute Nitric Acid	Continuous	Craig /HAFTA
*Hot Water	Continuous?	Wyman/Dartmouth, Antal/Hawaii Natural Energy Institute
*Hot Water-pH Neutral	Continuous?	Ladisch/Purdue
*Ammonia	Percolation	Lee/Auburn
*FIBEX	Continuous	Dale/University of Michigan
*Lime	Continuous?	Holtzapple/Texas A&M
*Wet Oxidation	Percolation	Lehrburger/Pure Vision
Peroxide	Percolation	
*Organosolv (Clean Fractionation)	Continuous	NREL

*Champion-based pretreatment technology

To facilitate gathering the type of information required for the second and third screens on the many pretreatments for which literature information is unavailable, a questionnaire was developed in the last month of reporting period. This questionnaire has been sent to the various technology developers/champions and results will be reported in the next reporting period. In cases where insufficient information is available for a particular pretreatment technology to pass the second screen, the technology will be rejected for NEP's use. However, any technologies rejected by NEP will remain under consideration by the Advanced Pretreatment Project for reevaluation as a longer-term pretreatment option. Pretreatments passing the second screen will be subjected to techno-economic analysis and evaluated in terms of their availability for near-term piloting and demonstration and intermediate-term licensing by a third party interested in commercializing them as part of an integrated process.

Fermentation Strain Selection

Another Stage 2 objective is to assess what fermentation strains warrant inclusion in the Stage 3 experimental process development phase of the project. During the reporting period, we initiated a literature survey to review published information about the performance characteristics and other attributes of potentially available ethanologenic fermentative strains. The performance characteristics that need to be understood to be able to select which fermentation strain is best suited for a corn stover conversion process include sugar utilization range, tolerance to corn stover hydrolyzates, and general robustness under saccharification/fermentation processing conditions using the process configuration ultimately shown to be the most economically attractive. Beyond this, the

strain must be available for licensing by a third party and for performance verification by NREL.

An extended survey of potential fermentation strains completed using the publicly available literature as well as in-house expert knowledge identified 32 potential strain options, some of which were based on using two fermentative microorganisms, either simultaneously or in series, to ferment the major hexose (i.e., glucose) and pentose (i.e., xylose) sugars present in corn stover hydrolyzates. This initial list of potential strains was then subjected to an initial screen to identify those strains proven or believed to be capable of achieving ≥80% ethanol yield on total sugars and producing a final ethanol concentration ≥4% w/v. Strains for which less information is known but which have been reported to possess other compelling traits (e.g., higher temperature fermentation or secretion of endoglucanases, both of which could lower the required cellulase enzyme loading) were also carried through this initial screen.

The 15 strains or strain combination options that passed this first screen were then evaluated to assess the impact of their reported or assumed substrate utilization range characteristics on the projected ethanol production cost, i.e., as calculated using our benchmark ASPEN Plus-based techno-economic corn stover conversion model which assumes that the fermentative microorganisms achieves 92% of theoretical ethanol yield from glucose; 85% of theoretical ethanol yield from xylose; and no ethanol production from galactose, mannose, or arabinose. The top 7 candidate strains or strain combinations based on the potential for additional cost savings due to increased substrate utilization range are listed in the table below. It should be noted that the Erwinia and Klebsiella strains are distinct from the other strains in secreting endoglucanase enzymes and having the ability to directly utilize cellobiose, a potent feedback inhibitor of fungal cellulases. The economic and other attendant benefits of such attributes need to be characterized to properly assess their full potential.

Candidate Fermentation Strain Options

Strain ID	Strain Developer /IP owner
Escherichia coli KO11 & SL40	L.O. Ingram/BC International
Escherichia coli FBR3 (pLO1297)	L.O. Ingram/BC International
Saccharomyces cerevisiae and *Pichia stipitis* (wild type strains)	public strains
Saccharomyces cerevisiae 424A (LNH-ST)	N. Ho/Purdue
Erwinia chrysanthemi pLOI 555	L.O. Ingram/BC International
Klebsiella oxytoca P2	L.O. Ingram/BC International
Zymomonas mobilis AX101	M. Zhang/MRI

As mentioned above, there are important issues beyond sugar utilization efficiency that influence strain selection, especially tolerance to hydrolyzate and the ability to perform on low-cost nutrients. In addition to being able to verify that the strain performs well and is generally robust for the intended application, the strain also must be available for licensing by a third party interested in commercializing the process technology. The literature review shows that there is scant information about the nutritional requirements

for all of these strains, especially on corn stover hydrolyzates. Thus, developing low-cost nutritional supplements is a key area that needs to be experimentally explored for any of the strains that end up being carried into the Stage 3 development effort.

The next step in terms of identifying which strains should be considered for Stage 3 is contacting the various strain developers or owners/licensees to determine the availability of their strain(s) for third-party licensing and performance verification. If the strain(s) are available, we will determine what additional information is available about nutrient requirements and strain scale up. This effort of contacting the various strain developers/owners to clarify strain availability and performance attributes is currently underway and will continue into early FY02.

There are several potential process configurations under consideration: sequential (enzymatic) hydrolysis and fermentation (SHF); simultaneous saccharification and fermentation (SSF); and hybrid (enzymatic) hydrolysis and fermentation (HHF). HHF represents the case where enzymatic hydrolysis begins at a high temperature without fermentation and then, after a specified amount of time or extent of cellulose conversion, the temperature is reduced and the system is inoculated and proceeds to completion in an SSF mode. We anticipate using an HHF process configuration for saccharification and fermentation, since existing fermentative microbes are already considerably less thermotolerant than cellulase enzymes and further increases in enzyme thermotolerance are being targeted by the enzyme developers. However, we are also exploring the possibility of using a separate hemicellulose hydrolyzate fermentation in the context of co-location with an existing corn mill ethanol plant and in this scenario using a combination of *P. stipitis* and *S. cerevisiae* may be advantageous. Clearly, the process configuration we choose will also affect which ethanologen is ultimately selected.

Subcontractors

K. Paustian, National Resource Ecology Laboratory
XCL-0-30099-01
Modeling the Effects of Corn Stover Collection on Soil Carbon Sequestration and Other Aspects of Soil Sustainability (in support of the Corn Stover to Ethanol Life Cycle Analysis)
8/29/00 - 3/28/01
Completed as described in previous reporting period. Future work is anticipated in this area but will be carried out under a new subcontract.

R. Nelson, Kansas State University
ACL-0-30023-01
Water and Wind Erosion Models
3/30/00 - 11/30/00.
Completed as described in previous reporting period. Future work is anticipated in this area but will be carried out under a new subcontract.

R. Helm, F. Agblevor, Virginia Polytechnic Institute & State University
Elucidate Overliming Mechanisms and Explore Alternative Methods
XCO-9-29049-01

7/31/00 - 3/31/02
Extended through the end of FY01 as described in the previous reporting period. Progress has been slowed by a delay in hiring staff to support the project. Hiring was completed during the performance period and an extension was put in place to enable task completion. We will re-compete this subcontract in FY02.

Biomass Agri-Products (B/MAP) LLC
XCO-1-31021-01
Identify and Overcome Pre-Conversion Processing Issues for Corn Stover at Pilot and Industrial Scales
02/01/2001 - 09/30/2004
B/MAP will prepare three test grades of washed corn stover that meet NREL specifications. Then a large batch of the grade exhibiting the best handling characteristics will be produced and supplied to NREL for pretreatment testing. The schedule for acquiring and installing washing equipment at the B/MAP facility will delay preparation of washed material until the end of FY01. Progress is about 6 weeks behind schedule, but this has not dramatically impacted work at NREL since repairs are still being made to our pilot scale pretreatment system.

cea, Inc.
ACO-1-31042-01
Innovative Methods for Collecting, Handling, and Transporting Corn Stover
06/27/2001-12/26/2001
cea, Inc. will investigate several innovative approaches to reducing the costs of corn stover collection and storage. A final report will be delivered to NREL that documents their analysis, including their preliminary estimates of the economics of the proposed approaches. Recommendations will be made regarding future work priorities that will help to further decrease the cost of delivered corn stover to a bioethanol or biorefinery processing plant.

Rapid Analysis Project

Milestone Progress/Completion

C Milestone #227 – "Preliminary Rapid Analysis Method for the Chemical Characterization of Corn Stover Process Intermediates" (5/31/01) was completed on schedule.

Progress Highlights and Issues

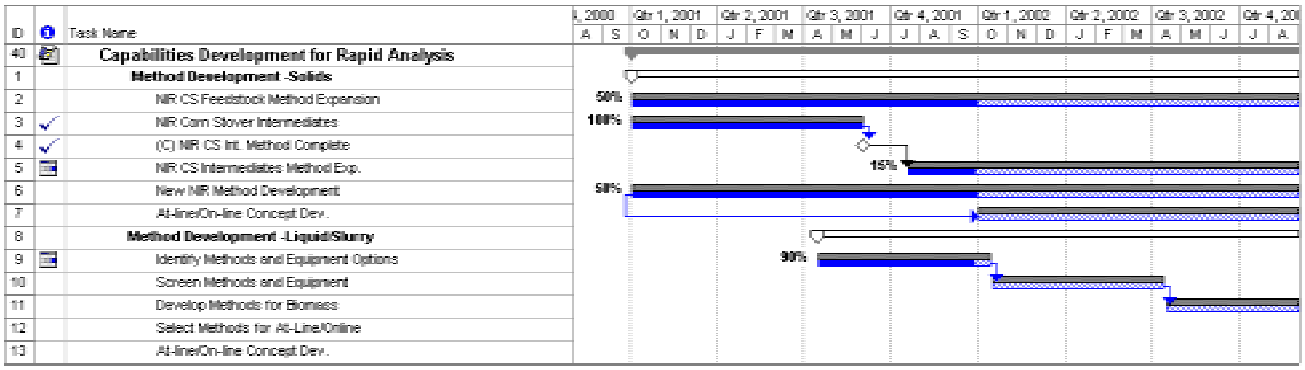

Figure 7: Rapid Analysis. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date shown is 9/28/01.

Method Development - Solids

NIR Corn Stover Feedstock Method Expansion

The rapid analysis method for the chemical characterization of corn stover will soon be expanded to include samples from five new locations and samples representing 13 known varieties of commercially grown corn. Corn stover samples representing six different anatomical tissues will also be included in the next method expansion. Chemical characterization of these corn stover samples was delayed as wet chemical method were improved and validated. All NREL laboratory analytical procedures (LAPS) are being revised to document these improvements. A more complete compositional analysis of both the new and old calibration samples will soon be completed. These improvements should allow better correlation with the NIR spectra and will expand the PLS predictions to include additional biomass components. An improved method should be available early in FY2002. The outlier identification flags associated with the stover equation have been used to identify samples with unusual glucan, xylan, lignin and protein contents. Flagged samples are used to expand the current calibration range. These new files were used to screen approximately 3,000 Colorado grown corn stover samples for interesting compositional mutations.

NIR Corn Stover Intermediates Preliminary Method Development

Thirty-nine samples from pretreatment experiments using different reactors at varying severities were used to calibrate a preliminary NIR/PLS (partial least squares) method for the chemical characterization of corn stover process intermediates. Chemical compositional analysis was performed on each of the calibration samples using standard wet chemical techniques to determine the levels of lignin, glucan, xylan, arabinan, galactan, mannan, protein, extractives, and ash. A PLS equation was obtained that predicted compositional chemistry from the NIR spectrum with errors that closely matched those observed for the wet chemistry used in the method calibration.

The method is designed for grab-sample analysis and requires only simple sample preparation. The new NIR/PLS rapid analysis method was successfully validated with a set of 15 independent samples with chemical compositions reflecting the entire

calibration range. The new NIR/PLS rapid analysis method for determining the chemical composition of corn stover process intermediates will be used to provide a quick estimate of the composition of pretreated corn stover. These quick estimates are being used to set enzyme loadings for SSF experiments. For at least six months, the compositional estimates of the preliminary method will be confirmed by standard wet chemical methods. The preliminary method will be improved and expanded as described earlier for the stover feedstock methods. A few samples of SSF process residues for corn stover have been received and will be included in future models to expand the calibration range.

NIR Corn Stover Intermediate Method Expansion

All solids produced in NREL projects utilizing corn stover for fuels and chemical production will be screened for use in improving and expanding the stover process intermediate calibration. Solids produced by members of the CAFI Pretreatment Consortium have been received and are being evaluated for use in method expansion and improvement.

New NIR Methods Development

Work continues to improve and expand the wet chemical characterization methods that are used to calibrate the rapid analysis methods. As data becomes available, new rapid analysis methods will be available to support research efforts. Recent accomplishments include changes in the method used for determining acid soluble lignin and the addition of methods for analyzing non-structural sugars, structural protein and chlorophyll. Procedures have been modified to differentiate soil and structural inorganics. Development of a new method for determining uronic acid groups in hemicelluloses has continued. New NIR methods for rapidly determining these constituents will be included in all updated corn stover feedstock methods. Calibration samples have been identified and data has been collected for developing a new NIR method for determining heating value in process residues.

At-Line/On-Line Concept Development

Representatives from Foss NIR Systems presented a seminar at NREL on on-line instrumentation on May 15, 2001. Foss industrial instruments may be suitable for the majority of samples produced in the PDU. Foss NIR systems has offered to provide NREL with industrial instruments for evaluation and testing.

Work continues to understand the challenges of the more difficult transfers we envision in the future as calibrations are moved from bench-top instruments to on-line and field-mobile spectrometers. The focus during this performance period has been on transferring spectral and calibration files between multivariate analysis software packages. Being able to perform transfers in this manner eliminates the need to duplicate the expensive and time-consuming process of method development for each new instrument. Eventually, these rapid analysis calibrations will provide a valuable resource for technology transfer and a tool that can be used to facilitate a wide range of biomass utilization technologies.

The ability to transfer calibrations between instruments enables us to efficiently share calibration methods that we have developed with outside collaborators like ORNL and

industrial partners like BC International. It also enables us to incorporate and utilize calibration methods developed by other groups like Pioneer Seed and Coors.

Method Development – Liquids / Slurries

Identify Methods And Equipment

Information has been collected on instruments for NIR, Fourier transform infrared (FTIR) and Raman spectroscopy from Ocean Optics, Bruker, Yamato Scientific, Foss North America, Nicolet, Analytical Spectral Devices, Perkin Elmer, Applied Spectral Systems, Jasco, and Perten. This information includes instruments for solids analysis and field analysis as well as for the analysis of liquids and slurries. A literature review of these commercial instruments and accessories will be completed early in FY02. Foss NIR systems has offered to provide NREL with liquid sample modules for evaluation and testing.

Support Of Other Research Projects

Near-term Ethanol Project
Rapid analysis methods can cost-effectively be applied to a large number of samples and thereby used to provide levels of information that have not been previously available. For example, approximately 200 samples from multi-year storage study were analyzed using the NIR/PLS stover feedstock method. Analysis of all samples collected from rounds 1-5 of the storage study provided a more complete data set for the ANOVA assessment of compositional changes in corn stover during storage. Comparison of the NIR/PLS analysis with wet chemical analysis of the storage study samples provided insight into the limitations of both methods. This information was used to guide improvements in both areas.

Advanced Pretreatment Project
The rapid analysis method for the chemical characterization of hardwood process intermediates was used as a preliminary screen for all pretreated corn stover samples. This preliminary assessment allowed SSF assessment of pretreated material to proceed within hours of completing pretreatment.

Member of the Biomass Refining CAFI were trained at NREL in biomass compositional analysis using the improved wet chemical methods.

Engineered Feedstock Project
Eighteen distinct samples of corn stover with known properties have been analyzed to assess the effect of genetic composition and location on corn stover composition. The analysis of more than 200 individual corn stover samples has provided a more accurate understanding of corn stover composition and natural compositional variance. Similar studies would be too costly to pursue without the savings in compositional analysis time and cost that the rapid analysis method provides. The rapid analysis screening allowed the completion of the 8/30/2001 C-milestone.

BC International CRADA
In support of the NREL/BCI cooperative research and development agreement (CRADA), rapid analysis project team members continue to work with BCI to validate NIR/PLS methods for the chemical characterization of bagasse and rice straw feedstocks as well as process intermediates from the BCI bagasse conversion work. These methods could be used to calibrate at-line and on-line rapid analysis methods for use in BCI's biomass-to-ethanol processes.

External Analytical

Milestone Progress/Completion
None to report; 2 reported in 1st half FY01.

Progress Highlights and Issues
This task manages and coordinates external analytical chemistry support for the Ethanol Project. Two competitively awarded subcontracts for outside laboratory analytical support are currently active, one with Hauser Chemical Research and the other with

Industrial Laboratories. Initially placed in August 1999, both of these companies have since been validated by NREL to perform biomass compositional analysis based on standard NREL LAP's. When time or internal resources for analytical chemistry support are limited, samples are sent out to these external laboratories to expedite research progress. Periodic QA/QC checks are made by the NREL subcontract technical monitor to ensure that these laboratories produce high quality analytical data.

The 2 support laboratories analyzed a total of 30 solid biomass samples during the reporting period. (A higher proportion of samples were analyzed at NREL than normal during this period to enable improvements in the standard analytical methods for corn stover compositional analysis to be developed and validated.) Even with the reduced number of samples analyzed, applying periodic QA/QC checks continued to be critical to ensure that the subcontractors are providing Ethanol Project researchers with high quality compositional data.

The SOW for each of these subcontracts is currently being modified to expand the scope of the analytical methods as they are validated to run in order to enable the subcontractors to incorporate enhancements that were made to several of the NREL standard analytical procedures for corn stover analysis during the reporting period.

Subcontractors

Hauser Chemical Research
JDH-9-18127-01
Compositional Analysis of Biomass Samples
08/05/99 - 08/04/02
During the reporting period Hauser analyzed 30 biomass solids samples.

Industrial Laboratories
JDH-9-18127-02
Compositional Analysis of Biomass Samples
8/12/99 - 8/11/02
During the reporting period Industrials Laboratories did not analyze any biomass solids samples.

ASTM Activities

Milestone Progress/Completion

P Milestone #281 – "Report Documenting FY2001 ASTM E48.05 Subcommittee Accomplishments and Plans for FY 2002" (9/30/01). Eight upgraded methods were presented to ASTM for the September ballot. The ballot is open until October 1, 2001. The report was submitted as scheduled.

Progress Highlights and Issues

Standard Methods

In 1995, NREL published eight methods for characterizing biomass through American Society for Testing and Materials (ASTM). These methods were revised and submitted to ASTM for renewal in the September 2001 ballot.

- E1690-95 The determination of ethanol extractives in biomass.
- E1721-95 The determination of acid-insoluble residue in biomass.
- E1755-95 The determination of ash in biomass.
- E1756-95 The determination of total solids in biomass.
- E1757-95E01 The preparation of biomass for compositional analysis
- E1758-95E01 The determination of carbohydrates in biomass by HPLC.
- E1821-96 The determination of carbohydrates in biomass by GC.

Three additional methods must be validated and written before a total mass closure method can be submitted for the analysis of wood. These methods are:

Determination of acid soluble lignin
Determination of uronic acids
Determination of acetyl and formyl groups

Methods for acid soluble lignin and acetyl/formyl groups have been prepared and are currently in use at NREL. Drafts of new ASTM methods are in preparation and will be included in the January 2002 ASTM ballot. A preliminary method for uronic acid determination is currently in development and data collection to support a new ASTM method will begin in October 2001. A method for the complete compositional analysis of biomass will be submitted for ASTM ballot after all 11 of the individual methods have been published.

NIST Standard Reference Materials

The ASTM E48.05 subcommittee successfully completed the paperwork necessary to release four standard reference materials (SRM) prepared at NREL in 1989. These materials have been released and are currently available for purchase directly from the National Institute of Science and Technology (NIST). Reference numbers for these standards are 8491 bagasse, 8492 poplar, 8493 pine, and 8494 wheat straw.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

- C-milestone presentation at NREL: "Preliminary NIR/PLS Method for the Rapid Chemical Characterization of Corn Stover Process Intermediates." B. Hames*, A. Sluiter, C. Roth, D. Templeton, R. Ruiz., NREL, September 6, 2001

Scientific Meetings: Papers/posters presented or recently accepted for presentation

- Schell, D.; Sáez, J.; Hamilton, J.; Tholudur, A.; McMillan, J. 2001. Use of Measurement Uncertainty Analysis to Assess the Accuracy of Carbon Mass Balance Closure for the Cellulase Production Process. *Appl. Biochem. Biotechnol*. Submitted. (Presented as poster 3-26 by D. Schell at the 23rd Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO, May 6-9 [2001].

- Templeton, D.; Hames, B.; Thomas, S.R.; McMillan, J. 2001. Sources of Variability in Corn Stover Composition. Poster 1-10 (retitled) presented (by D. Templeton) at the 23rd Symposium on Biotechnology for Fuels and Chemicals, May 6-9, 2001, Breckenridge, CO.

- Newman, M.M.; Dowe, N.; McMillan, J.D. 2001. Comparing the Efficacy of Commercially Available Cellulase Enzyme Preparations for Complete Cellulose Hydrolysis via Simultaneous Saccharification and Fermentation. Poster 2-14 presented (by M. Newman) at the 23rd Symposium on Biotechnology for Fuels and Chemicals, May 6-9, 2001, Breckenridge, CO.

- Hames, B.; Havercamp, A.; Roth, C.; Meglen, B. 2001. Rapid biomass analysis: New analytical methods supporting ethanol production from biomass. Oral presentation and poster presentation at the 23rd symposium on Biotechnology for Fuels and Chemicals, May 6-9, 2001, Breckenridge, CO.

- McMillan, J.D. 2001. What Does it Take to Make a Commercial Enzyme Process Work? Presented at California Institute for Food and Agricultural Research (CIFAR) Conference XIV on June 4th, 2001, in Davis, California.

- McMillan, J.D. 2001. Status of Enzymatic Hydrolysis of Biomass to Produce Ethanol in the United States of America. Presented at the International Seminar on Biomass for Energy Production (INTERBIO), June 28-29, 2001, Rio de Janeiro, Brazil.

- McMillan, J.D. 2001. Update on the USDOE's Biofuels and Biorefineries Projects. Presented at Centro de Technologia Copersucar, Piracicaba, SP, Brazil, July 2, 2001.

- McMillan, J.D. 2001. Cellulase Enzyme Production and Efficacy Evaluation Research at NREL. Presented at FAENQUIL, Lorena, SP, Brazil, July 5, 2001.
- McMillan, J.D. 2001. Ethanol Production from Starch and Cellulosic Biomass: Status and Prospects. Presented at the Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil, July 10, 2001.

- McMillan, J.D.; Kadam, K.L. 2001. Fermentation Process Integration Using Separate or Simultaneous Cellulose Saccharification. Paper (S53) presented at the Society of Industrial Microbiology annual meeting, July 29-August 2, 2001, St. Louis, Missouri.

- Hames, B. R.; Sluiter, A. D.; Roth, C. J.; Meglen, R. R. 2001. New Analytical Methods Supporting Fuels and Chemical Production from Biomass. Oral presentation at the Fifth Biomass Conference of the Americas. Meeting originally scheduled for Orlando, Florida, September 17-21, 2001, meeting postponed new date TBD.

- Kelley, S.; Hames, B. R; Meglen, R. R. 2001. Use of Near Infrared Spectroscopy for Characterization of Wood. Poster presentation at the Fifth Biomass Conference of the Americas. Meeting originally scheduled for Orlando, Florida, September 17-21, 2001, meeting postponed new date TBD.

Scientific Journals: Papers accepted for publication

- Sokhansanj, S.; Thurhollow; Kadam, K.; McMillan, J. 2001. Stalking a new fuel source. Resource **8**(5): 11-12 [May 2001]. (ASAE publication)

- "Chemical Analysis of Wood Chips in Motion Using Mid-Infrared Spectroscopy with PLS Regression." R. W. Jones,*, R. R. Meglen, B. R. Hames, and J. F. McClelland, accepted for publication in *Analytical Chemistry*, September, 2001.

STRAIN RESEARCH

Summary of Technical Achievements or Results

The Strain Research Team during this reporting period has focused on following four research activities:

1) Arabinose-fermenting yeast: We initiated a comprehensive study of L-arabinose transport in yeast. *Saccahromyces* appears to show low L-arabinose transport activities. Uptake studies have identified some yeast species that have better L-arabinose transport than *Saccharomyces*. In addition, we also started a classical mutagenesis approach.

2) Evaluate second generation ethanologens: Thirty-two strains of Lactobacillus were selected for evaluation based on the criteria established for yeast. These criteria are: hydrolysate (corn stover) tolerance, temperature tolerance, ethanol tolerance, and fermentation at low pH. One of strains, *L. rhamnosus* performed fairly well under all conditions tested.

3) Improve *Zymomonas*: We have successfully developed the integration methods in a more robust host strain *Z. mobilis* 31821 and demonstrated that xylose-fermenting genes can be integrated into the genome of *Z. mobilis* 31821. A fully integrated *Zymomonas* 31821 strain is able to grow on xylose medium and ferment xylose to ethanol. The growth rate on xylose is relatively low, however, now that the integration methodology is established we believe that we can develop improved strains for xylose utilization.

4) Yeast platform: Thirty strains of yeast were evaluated for fermentation performance in various levels of corn stover hydrolysate and temperatures. Three strains demonstrated robust characteristics in hydrolysate and elevated temperature and meet two out of the four selection criteria for a robust yeast strain based on the initial screening protocols. We recommend continuing the effort to screen candidate strains based on the last two criteria, low pH and high ethanol resistance. A series of three colloquy meetings were held in Denver, Chicago, and Washington, DC, with stakeholders of our Yeast Platform Project. The goal of these meetings was to seek expert input, examine the project hurdles, and discuss issues and concerns regarding our current vision for the project. More than 40 people representing 20 companies and 12 universities as well as USDA and ORNL participated the colloquies.

General Technical or Scientific Progress

Arabinose Fermenting Yeast Project

This is a funds-in CRADA with CRA/NCGA. The goal of the project is to develop new yeast biocatalysts that are capable of fermenting L-arabinose from corn fiber into ethanol. During this period, we focused on mutagenesis of the engineered yeast strain and determining the capability of yeast cells to transport L-arabinose.

Milestone Progress / Completion

C Milestone #228 – "Demonstrate the Feasibility of Conversion of L-arabinose to Ethanol by Expression of Bacterial *ara* Genes in *Saccharomyces Cerevisiae*" (9/30/01). A comprehensive study of L-arabinose transport in yeast has been initiated. Uptake studies have identified some yeast species that have better L-arabinose transport than *Saccharomyces*. In addition, we also started a classical mutagenesis approach. The C-milestone report was completed on schedule.

P Milestone #286 – "Review Meetings with CRA/NCGA Technical Review Panel" (9/30/01). Quarterly meeting is scheduled with the CRA/NCGA scientific board. We made two presentations (in Breckenridge on May 8 and in Chicago on August 14, 2001) to the CRA/NCGA scientific board during this period. This P-milestone is completed.

Progress Highlights and Issues

We used chemical (ethyl methane sulphonate, EMS) mutagenesis in an attempt to isolate mutants of the engineered strain that would grow on L-arabinose. We mutagenized one of the original strains (BFY013) and one of the newly engineered strains (BFY064). We have screened approximately 6 x 10^9 mutagenized cells for growth on L-arabinose. Thus far no desired mutants have been discovered.

We were successful in obtaining 14C-labeled L-arabinose. We set up the equipment, protocols, and a laboratory for studying L-arabinose transport using this radioactive sugar. A comprehensive study of L-arabinose transport in yeast has been initiated.

The engineered *Sacchromyces* strain grown on galactose showed both high and low affinity galactose transport. These same cells had a very low affinity L-arabinose transport activity. L-arabinose uptake was much lower than galactose uptake. These experiments also demonstrated that our indirect method for assessing uptake levels was valid. The arabinose transport results indicate that the engineered strains are not expected to grow at usual sugar concentrations even if all the enzyme activities are expressed at sufficient levels. We may have to isolate an efficient L-arabinose transporter from a different organism and engineer it for expression in *Saccaromyces*. For this purpose, we have begun to identify L-arabinose transport in other yeast species. We have found that one strain showed a low affinity arabinose uptake but a high capacity for sugar uptake. This capacity for arabinose transport appears to be about 10-fold higher than that in *S. cerevisiae* strains. We are continuing to investigate the L-arabinose transport kinetics for other strains.

We proposed a future work plan to the CRA/NCGA technical review board. The CRA/NCGA is planning to renew the current CRADA for a third year.

New Commercial Ethanol Strains

Milestone Progress / Completion

P Milestone #339 – "Renew all Subcontracts in New Commercial Ethanol Strains" (5/30/01). All four subcontracts are in place.

Progress Highlights and Issues

Subcontractors

L. Ingram, University of Florida/BC International
ZDH-9-29009-04
Development and Commercialization of New Ethanol Producing Strains -
"Develop of portable ethanol-production operons for expression in Thermotolerant gram-positive bacteria"
7/15/99 - 9/30/01
During the past six months, two new genes have been identified in Gram-positive bacteria that may encode pyruvate decarboxylases (PDC). The amino acid sequences of these genes are very different from the yeast and Z. mobilis PDC genes but were readily found using our newly described sequence for the Sarcina ventriculi gene. One of these putative PDC genes is in Lactococcus lactis and the other is in Clostridium acetobutylicum. During the course of this work we also noted that sufficiently close evolutionary relationships exist between indole-3-pyruvate decarboxylases and pyruvate decarboxylases that true physiological activity cannot be assigned without direct experimentation. Accordingly, we are cloning both. The new C. acetobutylicum gene has now been been cloned and is expressed at very high levels in E. coli. However, most of the product is found as an insoluble inclusion body. We are currently attempting to assay the soluble fraction for both decarboxylase activiites, refolding if required. Additional studies have also completed the kinetic characterization of our recently cloned Zymobacter palmae PDC, the Acetobacter PDC, the Sarcina PDC, and the Zymomonas PDC (for comparison). Two papers are in press and appear soon, one in the British journal entitled Microbiology (Sarcina), and the other in Archives of Microbiology (Acetobacter). A third paper describing kinetics and cloning of Zymobacter PDC is in preparation. The Sarcina PDC has been purified to homogeneity and attempts are now being made to crystallize the protein for a detailed structure analysis. Comparison of this structure to the yeast PDC and Zymomonas PDC should provide insight into the structural determinants of Km and provide opportunities for genetic modification.

A no-cost extension of the period of performance may be required, however once the funds are expended the subcontract will be closed out.

T. Jeffries, University of Wisconsin/Iogen
ZDH-9-29009-01
Development and Commercialization of New Ethanol Producing Strains -
"Metabolic Engineering of Yeasts for Commercial Ethanol Production"
12/31/00 - 12/30/01
This subcontract was a one year subcontract effort which was to be extended a second year. Due to difficulties of the subcontractor to find resources to continue this effort the Subcontractor and NREL decided not to pursue the second year funding.

H. Lawford, University of Toronto/Iogen
ZDH-9-29009-02
Development and Commercialization of New Ethanol Producing Strains

"Improve recombinant Zymomonas mobilis for efficient and rapid fermentation of hexose and pentose sugars"
7/15/00 - 12/31/01
Research carried out during the period compared the performance of strains C25 and AX101. A study on effect of acetic acid on xylose utilization by strain AX101 was also conducted. A modification to the subcontract was approved to add an additional $53,441 to the subcontract and extend the period of performance by four months to 12-31-01. The subcontract will be closed out once the funds are expended.

N. Ho, Purdue University
XCL-1-31040-01
"Further improvements to the effectiveness of genetically engineered xylose fermenting Saccharomyces yeasts in cofermenting glucose and xylose"
(6/6/01 – 5/5/02)
The research has been initiated in improving xylose transport. A method for generation of mutants in polyploid yeast strains is under development. We are expecting bimonthly technical reports.

Second Generation Ethanologens Project

The objective of this project during the reporting period was to perform a competitive analysis of selected microorganisms to yeast.

Milestone Progress/Completion

C Milestone #229 – "Evaluate microorganisms for 2nd generation ethanologen development and its competitiveness to yeast" (9/30/01). Thirty-two strains of *Lactobacillus* were chosen for evaluations of fermentation performance on CYA media in corn stover hydrolysates, ethanol tolerance, and performance at different temperatures and pHs. The work and the milestone report were completed on schedule.

Progress Highlights and Issues

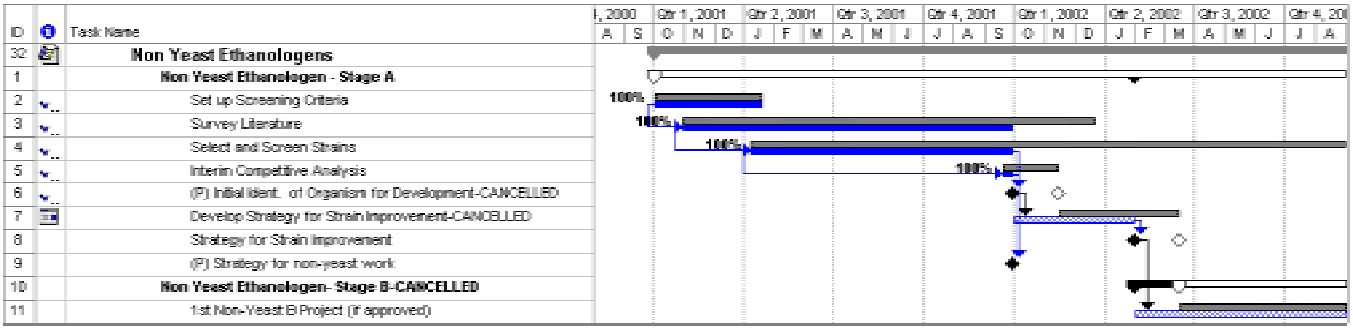

Figure 8: Second Generation (Non- Yeast) Ethanologens. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date shown is 9/28/01.

Our goal for this project was to screen and select non-yeast microorganisms for development as a future generation ethanologen based on criteria established by the yeast platform project and then to determine its competitiveness with yeast. An important consideration in this screening effort involved establishing boundaries for this evaluation.

Non-yeast microorganisms encompass a large number and variety of strains, some of which may not be bacterial. The focus of this project was directed towards Lactobacillus based on its availability and our experience with these organisms; with the understanding that other microorganisms should also be given serious consideration for development as future generation ethanologens.

Thirty-two strains of Lactobacillus were originally chosen for our evaluations, based on their ability to tolerate temperatures up to 45°C. Using in-house strains of Lactobacillus, obtained mostly from the American-type Culture Collection (ATCC), a preliminary screen was performed to assess growth in the presence of a lower nutrient medium than they are accustomed to. Fifteen out of thirty-two strains grew in CYA medium (2% glucose, 1% corn steep liquor (CSL), 0.05% yeast extract, and 0.25% ammonium sulfate) after passage through a richer medium (MRS). Of these, only 13 survived repeated passages through this medium. In general, strains from the same species behaved similarly.

Eight representative strains from all species that grew were selected for further evaluation of growth (glucose utilization) in neutralized corn stover hydrolysate. Additional evaluations in the presence of ethanol at 5%, 7.5%, and 10% ethanol (w/v) were performed and at several temperatures (37°C, 42°C, 45°C and 50°C). Of these strains, the *L. rhamnosus* species performed fairly well under all conditions tested. Only one strain (ATCC #10863) was selected for further analysis of pH effects in controlled fermentors. There are many strains of *L. rhamnosus* available that were not included in this evaluation, but could prove to be better hosts. Productivity of lactic acid correlated linearly to the pH of the medium.

Subcontractors

P. Rogers, University of New South Wales
XXL-9-29034-03
Second Generation Organism Development
8/5/99 - 8/4/01
This subcontract focused on screening thermophilic microorganisms from nature that can be developed for ethanol production. Samples were taken from hot springs in Australia, volcanically heated water and soils from New Zealand, local commercial scale heated composts, and from sugar mill waste in Queensland. Isolates were obtained by enriching samples in 4% ethanol at 60°C on Basal Minimal Medium supplemented with yeast extract and xylose. One hundred eighty-eight isolates were obtained. The most promising isolates can grow in the presence of 5% to 10% ethanol at 60°C and were identified by ribosomal RNA sequencing analysis and all belong to the Bacillus genus, with several strains belonging to a unique species.

The second year of this subcontract focused on characterizing the most promising isolates obtained and developing genetic tools for introducing an ethanol pathway into selected thermophiles. Transformation systems have been developed for introducing exogenous DNA into various selected thermophiles. Expression vectors are in the process of being constructed by incorporating promoters isolated from libraries derived from various

thermophiles. Further characterization of two thermophiles, B10EXG and M10EXG, has been conducted. The main byproduct is lactate, along with small amounts of acetate and ethanol when grown in a fully defined medium supplemented with glucose, trace elements and vitamins. Growth appears to stall when the pH drops to 4.5. Sequencing of the lactate dehydrogenase gene is in progress as a target for the introduction of ethanol genes.

L. Ingram, University of Florida
XXL-9-29034-01
Second Generation Organism Development
7/15/99 - 7/14/01
Over 300 isolates have been obtained from Florida, Georgia, Nevada and northern California desert soil samples using a screening method developed to isolate hydrolyzate resistant, thermophilic, and xylose metabolizing strains. The second year of this subcontract focused more carefully on characterization of obtained isolates.

The most promising strains can utilize xylose, grow in the presence of 5% poplar wood hydrolyzate at 50°C at pH 5.0 and were identified by 16s rRNA sequence analysis as belonging to the Bacillus genus. In a determination to discover fermentation pathways, metabolic by-products were analyzed. The major fermentation produced by these isolates was lactate. Acetate and ethanol amounted to less than 10% of the total fermentation products in the presence of glucose and 20% when grown in xylose. Viability declined when pH of the medium fell below 4.5.

Improved *Zymomonas* Project

The goal of this project is to construct a genome-integrated xylose-fermenting *Zymomonas* in 31821 host since the fermentation evaluation of *Z. mobilis* strains indicated that plasmid-bearing, xylose-fermenting 31821 performed better than an integrated xylose- and arabinose-fermenting 39676 culture in the 50% (v/v) overlimed corn fiber hydrolysate.

Milestone Progress / Completion

C Milestone #331 – "Completion of Gate C review and Final Documentation of 31821 Integration" (9/30/01). We have successfully integrated both xylAxylB and tal/tkt operons along with antibiotic markers in the genome of *Z. mobilis* ATCC31821. The strain is capable of growing on xylose although the growth rate is relatively low. Further characterization of the fermentation performance is needed. The work and the milestone report were completed on schedule.

Progress Highlights and Issues

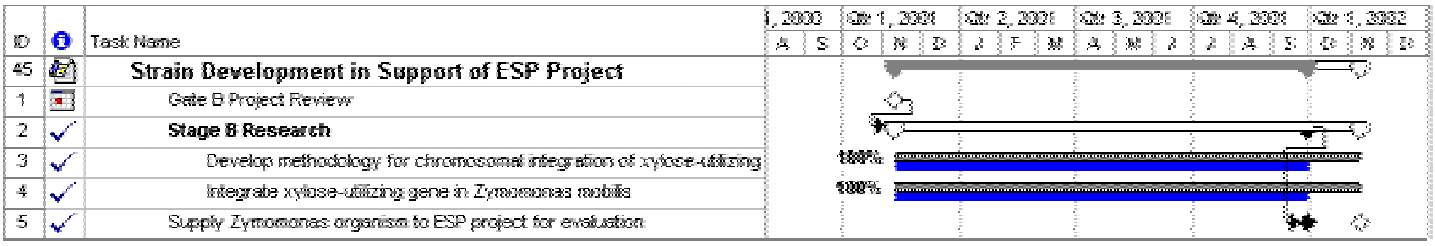

Figure 9: Improved Zymomonas Project. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01.

Effort has been continued on the developing a genomically integrated strain of *Z. mobilis* ATCC31821 for xylose utilization. Two integration systems are used in parallel to achieve this goal - homologous recombination and transposition. In the last six months, we have integrated the Pgap xylAB operon (half of the genes for xylose utilization) along with an antibiotic marker Tcr into ldh of *Z. mobilis* ATCC31821 using homologous recombination. Genetic analyses confirmed the events of integration. In the complementation experiments, the integrated strains were transformed with a plasmid containing Penotaltkt (the other half of the genes for xylose utilization) and were able to grow on xylose medium.

We also demonstrated that we could integrate all four genes required for xylose fermentation into the genome of 31821 by combining the two integration methods. However, we found that an integrant with Penotaltkt integration was not able to grow on xylose when Pgap xylAB was supplied on a plasmid as described in the last report. During this period, we made several improved version of the constructs. One of the strains, a fully integrated strain is able to grow on xylose medium and ferment xylose to ethanol. However, the growth rate is relatively low.

We have successfully developed the integration methods in a more robust host *Z. mobilis* 31821 and demonstrated that xylose-fermenting genes can be integrated into the genome of *Z. mobilis* 31821. Now the integration methodology is established and we believe that we can develop improved strains for xylose utilization.

Subcontractors

P. Rogers, University of New South Wales
XDH-0-29055-01
Improve Recombinant *Zymomonas Mobilis* for Efficient and Rapid Fermentation of Hexose and Pentose Sugars
8/5/99 – 12/31/01
Further characterization if integrant strain C25 with evaluation of acetate inhibition in batch culture as well as stability analysis in continuous culture were conducted. Work is continuing to develop integrant strains of ZM4 (pZB5) and ZM4 acetate resistant strain with pZB5. A no-cost extension is in place through 12/31/01.

Platform Yeast Project

The objectives of this project during this period are 1) to conduct a series of colloquies to bring the industry and academic experts together to further discuss the yeast platform project, solicit input, and discuss mechanisms to work together and 2) to evaluate yeast strains based on desired criteria for fermentation of biomass sugars

Milestone Progress / Completion

C Milestone # 302 – "Develop the "Vision" for the Yeast Platform Project" (4/30/01). This milestone was completed on schedule. The 'Vision" articulated in the milestone report was subsequently used in the yeast colloquies (see P Milestone #300 below) as the starting point for discussions.

C Milestone #230 – "Identify strain(s) best suited for a number of industrial processes/products" (9/30/01). Thirty yeast strains were evaluated in YCA media for their fermentation performance under various aeration conditions, hydrolysate tolerance, and temperatures. Three of the strains demonstrated superior performance with hydrolysate and high temperature. This milestone was completed on schedule.

P Milestone #301 – "Establish database for literature on industrial yeast, relevant biochemical pathways, and tools for strain development" (5/30/01). This milestone was completed on schedule and was described in the previous reporting period.

P Milestone #300 – "Seek opportunities to involve other key parties for collaboration on this project" (8/30/01). To identify key parties for collaboration on the yeast platform project, a series of colloquies were conducted in July to bring the parties together to further discuss the yeast platform project, solicit input, and discuss mechanisms to work together. This milestone was completed on schedule and the industrial consultant is preparing a report summarizing the colloquies.

Progress Highlights and Issues

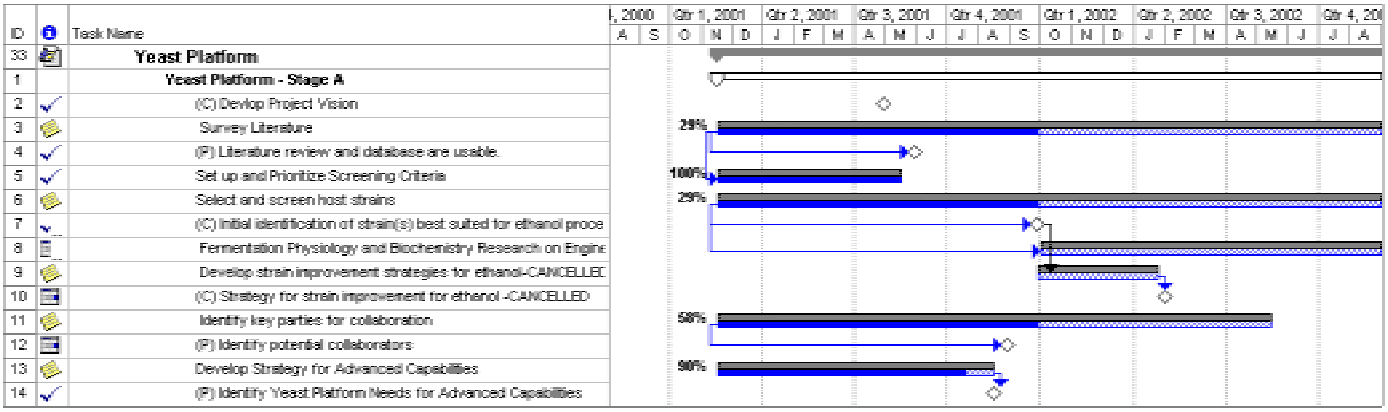

Figure 10: Yeast Platform Project. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01.

Fermentation criteria were listed and prioritized to determine the best yeast strain for a biomass to ethanol process. The criteria consisted of identifying a strain that

demonstrated one or more of the following characteristics: tolerance to acid-pretreated biomass hydrolysate, high temperature, low pH, and high ethanol concentrations. A literature search identified approximately 70 yeast strains, and 30 were quickly obtained for experimental evaluation. Three series of experiments were conducted: 1. Collect baseline fermentation data of strains in aerobic, microaerophillic, and anaerobic conditions; 2. Compare fermentation performance in neutralized, acid-pretreated corn stover hydrolysate; 3. Compare fermentation performance of strains in elevated temperatures.

Results from experiment #1 showed that aeration nearly doubles the cell mass generated in the fermentation, however, after glucose is depleted the cells quickly consume ethanol. Microaerophillic cultures had slightly higher cell mass production compared to anaerobic cultures and ethanol utilization decreased with decreasing oxygen supply. Several strains under anaerobic conditions showed slightly elevated levels of byproducts such as glycerol and lactic acid. Microaerophillic conditions were used in experiments #2 and #3.

The results of the corn stover hydrolysate evaluation show that 15 out of 30 strains utilized nearly all of the available glucose within 48 hours at 90% v/v hydrolysate concentration. The degree of tolerance to hydrolysate varies among these fifteen strains. Observation of the level of lag phase based on sugar utilization shows that four *S. cerevisiae* strains, one *Kluyveromyces*, and one *Candida* strain show the least amount of lag phase indicating higher natural resistance. Experiment #3 examined fermentation performance of yeast strains from 30°C to 42°C. Results showed that 9 out of 30 strains performed well up to 42°C. Four *Kluyveromyces* strains showed little or no response to the elevated temperature and performed the best out of the nine strains. Two *Saccharomyces* strains, two *Candida*, and one *Brettanomyces* also demonstrated higher tolerance to 42°C fermentations. Four additional strains of *Saccharomyces* demonstrated tolerance to 40ºC.

Three strains demonstrated robust characteristics in hydrolysate and elevated temperature and meet two out of the four selection criteria for a robust yeast strain based on the initial screening protocols. It would be highly recommended to continue the effort to screen candidate strains based on the last two criteria: low pH and high ethanol resistance.

We hosted three yeast platform colloquies across the country during July 2001. The goal of these meetings was to describe our current vision for the project and seek expert input, examine the project hurdles, discuss the issues, concerns, and suggestions of our stakeholders so that DOE and NREL can make informed decisions on the future direction of the project. More than 40 people representing 20 companies and 12 universities participated the colloquies. Representatives from USDA and ORNL were also present. Several people from DOE and NREL attended colloquies. Don Johnson, a Biobased Industrial Products Consultant, led the colloquies.

A synopsis of the three Yeast Platform Colloquies was summarized and distributed to the attendees for feedback. The inputs that are common to two or more colloquies are 1) Industry needs a yeast; 2) Intra- and inter-(Enzyme Sugar Platform) project integration and coordination is critical to success; 3) Sensitivity (economic) analysis and keeping

continuously updated are critical; 4) Hydrolyzate tolerance and pentose uptake are primary hurdles; 5) A synthetic hydrolyzate standard is needed to anchor as real life hydrolyzates evolve; and 6) Resources and timing are needed to achieve vision. The input from industry favored a program where NREL developed the scientific foundations of biomass sugar utilization and leveraged this knowledge in CRADA's with individual companies to develop specific strains for each commercial process.

Subcontractors

T. Jeffries, University of Wisconsin
XXL-9-29034-02
Second Generation Organism Development
7/7/99 - 4/30/03
No report during this period.

Scientific Publications, Presentations, And Other Activities

General Presentations /Travel

- We made two presentations of the technical progress of the NREL/CRA/NCGA CRADA on developing arabinose-fermenting yeast to the CRA/NCGA scientific board (one at Brekenridge on May 10, 2001 and one in Chicago on August 14, 2001.

- A series of 3 Colloquy meetings were held in Denver, Chicago, and Washington, DC, with stakeholders of our Yeast Platform Project. The goal of these meetings was to seek expert input, examine the project hurdles, and discuss the issues and concerns, regarding our current vision for the project. More than 40 people representing 20 companies and 12 universities as well as USDA and ORNL participated the colloquies.

Scientific Meetings: Papers/Posters Presented or Recently Accepted for Presentation

The following papers were presented at the 23rd Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO on May 8, 2001.

- "Investigations on the Bottlenecks in the Metabolic-Engineered *Zymomonas mobilis* Strains Through the Measurement of Enzyme Activities During the Batch Ethanol Fermentations." Qiang Gao, Min Zhang, James McMillan, Dhinakar Kompala.

- "Cofermentation of Glucose, Xylose, and Arabinose by Integrated Strains of *Zymomonas mobilis*." A. Mohagheghi*, K. Evans, Y.C. Chou, M. Zhang.

- "Performance of Immobilized *Z. mobilis* 31821 (pZB5) on Actual Hydrolyzates Produced by the Arkenol Technology." Tomiaki Yamada*, JGC Engineering. Inc., Yokohama, Japan; Michael A. Fatigati, Arkenol, Inc., Mission Viejo, CA; Min Zhang

Scientific Journals: Papers Accepted for Publication

None

ENZYME RESEARCH

Summary of Technical Achievements or Results

Cellulase technology research during this reporting period has focused on Cellulase Fundamentals, CBH I Expression, Cellulase Performance Assays, Non-Cellulase Enzymes, and Enzyme Subcontract Liason. Work on the Cellulase Fundamentals project was conducted at both NREL and Cornell University (Brady and Wilson). NREL researchers have constructed new variants of *A. cellulolyticus* EI, which incorporate systematic changes in surface chemistry so that trends in cellulose binding and hydrolysis can be elucidated. These data were analyzed and a manuscript describing the work was accepted for publication in *Appl. Biochem. Biotechnol*.

J. Brady at Cornell has completed the first computer modeling study of the water boundary layer recently discovered to reside above the surface of cellulose, and a manuscript describing this work has been submitted to *Biopolymers*. We expect this to be a landmark paper in the field.

In the CBH I Expression project, a multitude of fusion protein constructs have been designed and built to assess the possibility that *E. coli* can be used to express this important fungal enzyme. At year's end, all strategies to express the *cbh1*cd gene as fusion proteins, or co-express with disulfide bond forming proteins in Origami and/or BL-21 type strains of *E. coli* have failed to produce active, soluble protein. We thus conclude that *E. coli* cannot serve as an effective heterologous host for CBH I.

Cellulase Performance Assay research has focused on two general objectives: the benchmark testing of "state of the art" commercial cellulase samples from Genencor International and Novozymes; and the development of insights into the mechanism of cellulase action.

Work on the Non-cellulase project was focused entirely this FY on placing a subcontract with the University of Minnesota to conduct the research entitled "Lignin Depolymerase from *Trametes cingulata*."

Subcontract Liaison activities included organizing six research update meetings with Genencor International and three with Novozymes.

General Technical or Scientific Progress

Cellulase Fundamentals

Our goal was to study three well-defined aspects of cellulase biochemistry that pertain to improving cellulase performance and transfer new insights to the appropriate subcontractors and/or the cellulase research community through publications and presentations. The three aspects are outlined in the milestones below.

Milestone Progress/Completion

C Knowledge Milestone # 231 - "Learn about cellulase/cellulose interaction by conducting surface amino acid engineering on selected endoglucanase," (9/30/01). We completed this milestone on schedule by creating families of modified EI enzymes carrying mutations that varied surface chemistry and determining their cellulose interaction characteristics. This work, titled "Exploration of the Cellulose Surface Binding Properties of A. *cellulolyticus* Cel5A by Site Specific Mutagenesis" was accepted for publication in the peer-reviewed journal *Appl. Biochem. Biotechnol.*

P Milestone # 259 – "Learn more about protein structure rules that confer thermal stability to GH family 7 cellobiohydrolases, specifically *T. reesei* CBH I," (8/30/01). The objectives of this learning milestone were not met as planned. Our overall objective to develop a heterologous expression system for *T. reesei* CBH I have not been successful, thus we have not been able to test the 20+ amino acid mutations generated by computer modeling thought to confer enhanced thermal stability. However, we have modeled the structure of *T. reesei* CBH I using InsightII in an effort to better understand potential folding pathways of the protein. This learning milestone is an important element in the cellulase technology field and we wish to continue the work under a new P milestone in FY02.

P Milestone # 260 – "Learn more about thermodynamics of cellulase/cellulose interaction" (8/30/01). This milestone was met one month before plan and was captured in a manuscript recently submitted to the refereed journal *Biopolymers*. The manuscript is entitled "Computer Simulations of Water Structuring Adjacent to (1,0,0) Microcrystalline Cellulose Iβ Surfaces." This activity will continue in FY02 with an expanded set of objectives which build upon those accomplished this year.

Progress Highlights and Issues

Understanding the interactions between cellulases and cellulosic substrates is critical to the development of an efficient artificial cellulase system converting biomass to sugars. Molecular mechanics simulations have been used to model the structure of water adjacent to microcrystalline monoclinic cellulose Iβ surface at two different temperatures; 300K and 478K. Strong localization of the adjacent water was found for molecules in the first hydration layer, due to both hydrogen bonding to the hydroxyl groups of the sugar molecules and also due to hydrophobic hydration of the extensive regions of hydrophobic surface resulting from the axial aliphatic hydrogen atoms of the "tops" of the glucose monomer units. Importantly, significant structuring of the water was found to extend far out into the solution.

We hypothesized that the highly structured layers of water might present a substantial barrier to the approach of cellulase enzymes toward the surface in enzyme-catalyzed hydrolysis, and might significantly inhibit the escape of soluble products in dilute acid hydrolysis, contributing to the slow rates of hydrolysis observed experimentally. These observations are being used to aid the design of endoglucanases with improved performance on pretreated biomass. New cellulases from fungi and bacteria are also being discovered. Thermal tolerant exoglucanases are of special interest, because these enzymes would allow us to test elevated temperature saccharification concepts. During

the year, the genes coding two new exoglucanases and one new endoglucanase were discovered in *A. cellulolyticus* genomic DNA. The subcontractor is also making significant progress towards understanding cellulase action using molecular mechanics modeling.

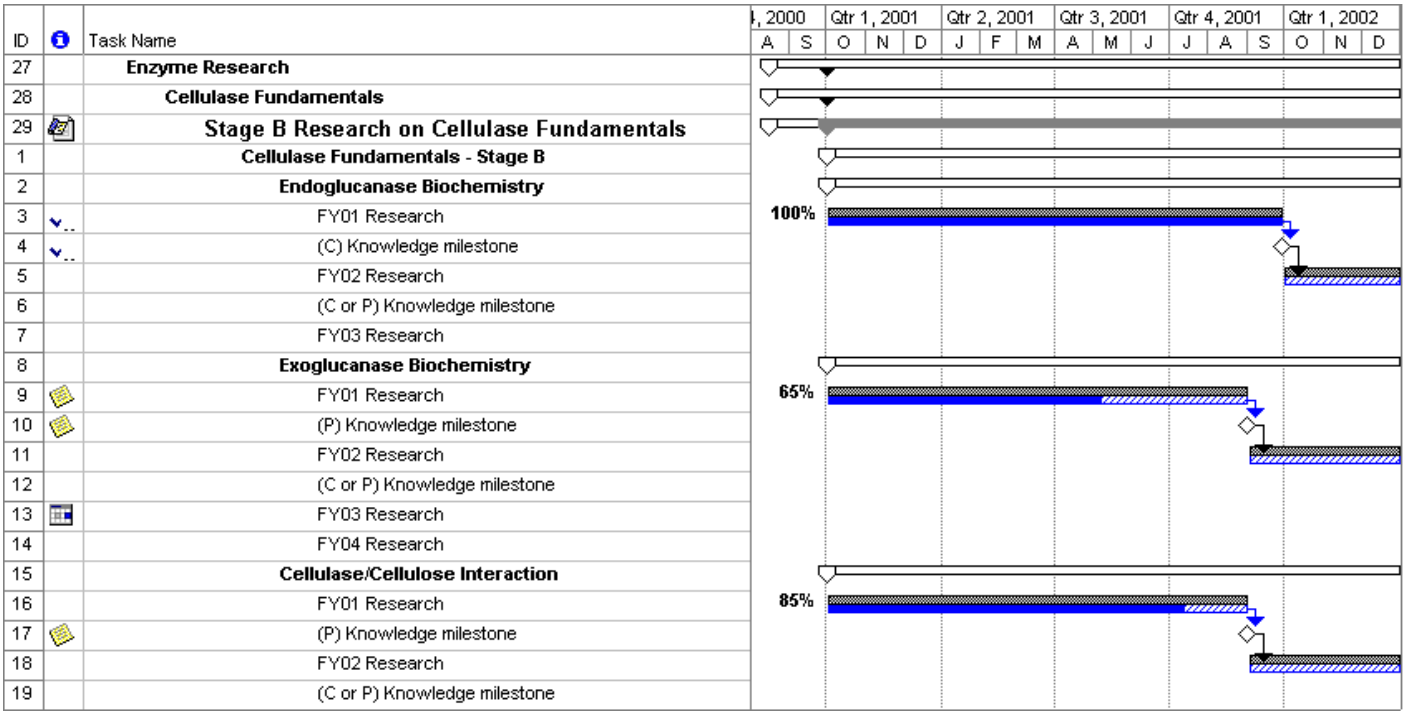

Figure 11: Cellulase Fundamentals Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01

Subcontractors

David Wilson, Cornell University
XDH-9-29048-01 mod 4
Improving *Thermomonospora fusca* Cellulase Enzymes by Protein Engineering
6/17/99-8/14/01 no cost extension 10/14/01

The subcontractor has been working to obtain Cel6A mutants that are more thermally stable and have higher activity on crystalline cellulose. For higher thermostability, family 6 sequence alignments were examined. Six sites for mutation were identified and these will be cloned for production of rCel6A. Each mutant will be tested for activity in the crude extract, along with its BMCC (bacterial micro-crystalline cellulose) activity, before purification. Only the modified enzymes that have good thermal stability or have higher activity on BMCC will be purified for further testing. Production of random mutations in Cel6A has also begun. Transformants will be screened for genes that confer changes in activity in Cornell's crystalline cellulose screen, but not in a CMC (carboxy methyl cellulose) screen. This may allow us to identify the residues required for crystalline cellulose activity.

John Brady, Cornell University
XDH-0-30009-01
XDH-0-30009-01 mod 2
Molecular Modeling of the Interaction of Cellulose with Cellulases and Catalysts
4/10/00-4/9/01
4/10/01-9/15/01
Molecular mechanics simulations have been used to model the structure of water adjacent to microcrystalline cellulose surfaces at 300^{o}K. Strong localization of the adjacent water was found for molecules in the first hydration layer, due to both hydrogen bonding to the hydroxyl groups of the sugar molecules and to hydrophobic hydration of the extensive regions of hydrophobic surface area due to the axial aliphatic hydrogen atoms of the "tops" of the glucose monomer units. Importantly, significant structuring of the water was found to extend far out into the solution. In the studies of the mechanism of *T. fusca* E2, molecular dynamics simulations have led to the proposal of a new mechanism for this protein that explains both the experimental mutagenesis data and the fact that it is an inverting enzyme.

Cellulase Assays

Our goal is to conduct validation and/or "benchmarking" studies of cellulase samples provided by subcontractor(s). Also, we are conducting research designed to better understand the mechanisms of cellulase action so that cellulase performance can be more accurately measured.

Milestone Progress/Completion

P Milestone # 268 – "Summarize Results of GCI Testing and Report to GCI/NREL," (8/30/01). This work was accomplished as planned. This project ("Integrated Task") was designed to provide Genencor International and Novozymes, Inc. with uniform, objective, and process-relevant measures of the effectiveness of submitted cellulase enzyme samples in the saccharification of lignocellulosic biomass. The project was charged first with establishing meaningful "baseline" measurements of the activity of the "best presently available commercial preparations," and then assaying subcontractor-submitted and NREL-generated modified enzyme mixtures by the same methods used for base-lining, in order to identify improvements in specific activity. A total of 22 subcontractor-supplied enzyme mixtures and/or purified enzymes have been subjected to a total of 124 diafiltration saccharification (DSA) experiments using pretreated yellow poplar as a substrate. Enzyme samples were assayed typically in triplicate (at minimum, in duplicate), with selected samples subjected to 6-10 level loading studies to determine dose-response for detailed comparisons.

P Milestone # 269 – "Develop Preliminary Stage of Cellulase Kinetic Time Course Model" (9/30/01). This work was accomplished as planned, but will be continued in a new P milestone next FY. Aspects of this study were presented at the Gordon Conference on "Cellulases and Cellulosomes" held in Proctor, NH, July 2001.

Progress Highlights and Issues

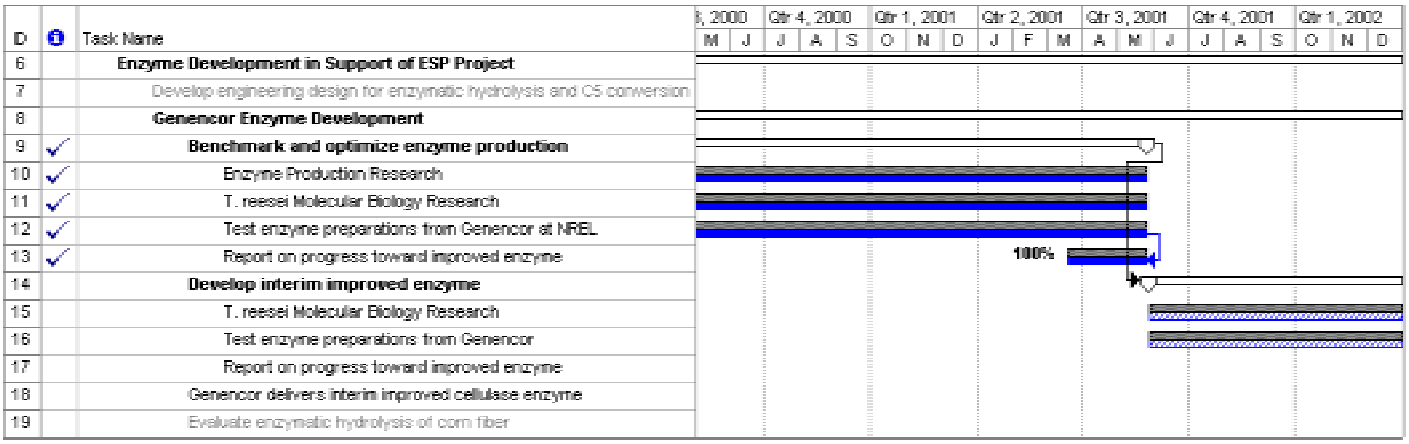

Figure 82: Cellulase Assay Support for Genencor Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01

A number of cellulase samples have been received from GCI and Novozyme for testing at NREL. Upon receipt, a portion of each sample was desalted by means of a standard size exclusion chromatography (SEC) treatment and characterized in terms of protein content by the Micro (Pierce) bicinchoninic acid (BCA) assay. A number of diafiltration saccharification assay (DSA) experiments have been carried out on these samples, including, at minimum, duplicate or triplicate standard assays of each. Selected enzyme mixtures have been studied more extensively by means of a total of four, 6- to 10-level dose-response studies. Results of these studies have formed the basis of six reports to GCI and two reports to Novozymes.

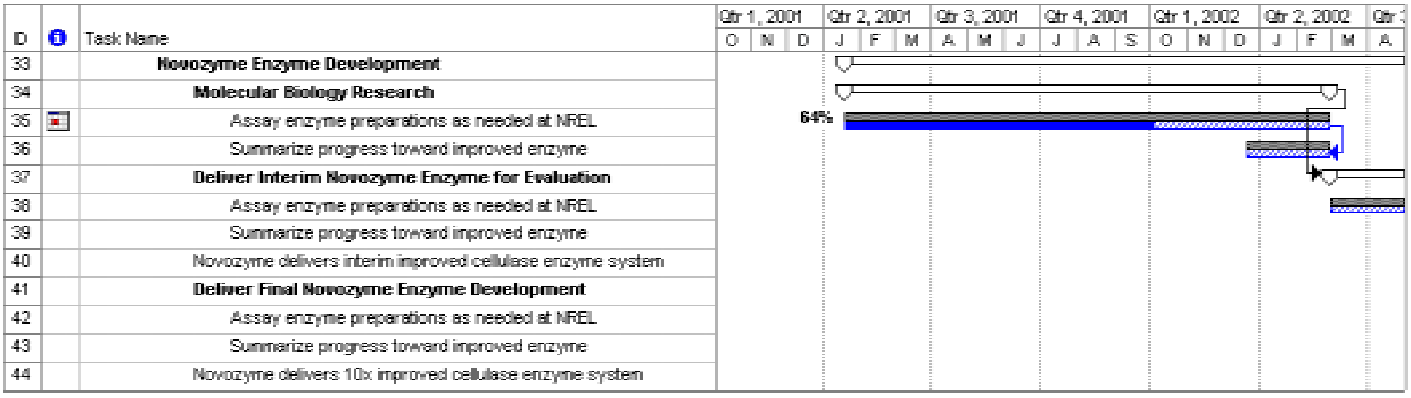

Figure 93: Cellulase Assays for Novozymes Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01

Subcontractor

Sharon Shoemaker, University of California at Davis
XDH-0-30009-03
XDH-0-30009-03 mod 1
Design and Test Improved Assays for Endoglucanases and Exoglucanases
6/1/00-6/1/01

6/1/01-8/15/01
New methods are being developed at UC Davis for characterizing cellulolytic systems and their individual components on the high molecular weight substrates, cotton cellulose, and *Acetobacter xylinum* cellulose. Both reducing sugars (RS) formed in the course of hydrolysis, and concomitant changes in molecular-weight distribution (MWD) of the high molecular weight substrates are being evaluated. The reducing sugars are being measured by 2,2'- BCA method while molecular-weight distributions are being obtained using high performance SEC combined with multi-angle laser light scattering (HPSEC-MALLS).

Research showed that cellulose molecular weight (MW) and degree of crystallinity determined the maximum amount of cellulose that could be dissolved and the time required for dissolution in DMAc/LiCl. Avicel (low MW, high crystallinity) and Solka Floc (high MW, low crystallinity) were dissolved at 6.0 mg/ml after ~8-h incubation with DMAc/8% LiCl at 50°C. Cotton cellulose (high MW, high crystallinity) could be dissolved only at 3 mg/ml following 48-h incubation at 50°C and 72-h incubation at room temperature. Bacterial cellulose, which had a chain length and crystallinity intermediate between Solka Floc and cotton, was dissolved at 6.0 mg/ml after ~12-h incubation.

High Throughput Screening (HTS) Project

Recent innovations in molecular biology have introduced new methods for improving the properties of enzymes. These techniques are based either on rational design using molecular mechanics modeling of protein structure or on random mutation of the gene coupled to numerically challenging selection and screening processes. Our goal is to develop HTS tools and strategies to improve selected cellulases, other biomass degrading enzymes, and metabolic enzymes.

Milestone Progress/Completion

K Milestone # 219 – "Acquisition, installation, and testing of the hardware necessary to conduct HTS and directed evolution of enzymes," (9/30/01). This milestone was met on schedule. A Zymark Staccato system was delivered to NREL on June 29th, 2001. The Factory Acceptance Test was carried out at Zymark in Hopkinton, MA from June 11th through the 15th and was observed by NREL scientists. Zymark Field Service Engineers installed the equipment in the Field Test Laboratory Building (FTLB) room 217 from July 16th to the 18th. Installation included validation and Site Acceptance Testing (SAT) of the equipment. The SAT protocol entailed re-teaching and validating the positioning of the plates by the arm to and from all of the instruments, demonstrating the dispensing and pipetting operating the SciClone, reading barcode labels, programming incubator operation including plate retrieval and storage, lid-handling, temperature maintenance, operating the plate reader, and demonstrating the software and data management capabilities of the system. The system consists of four basic subsystems; system control and data acquisition, incubation, liquid handling, and plate handling.

P Milestone # 262 – "Acquire HTS robotics." (6/30/01). This objective pertains to the specification and acquisition of a Zymark Staccato HTS system. The Zymark system was delivered to the NREL FTLB receiving area a day before June 30, 2001. The milestone was thus met as planned.

Progress Highlights and Issues

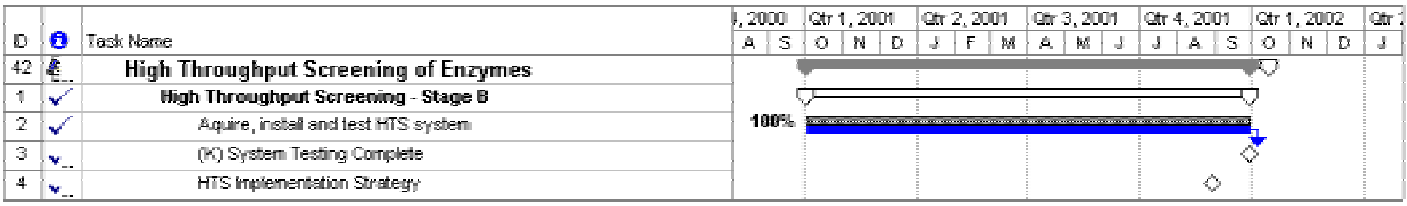

Figure 104: High Throughput Screening. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01

Modern methods have been developed to limit the mutations to the gene of interest and significantly enhance the mutation rate, allowing these changes to be screened in heterologous expression systems. The required screening is intensive; hundreds of thousands to millions of clones need to be screened for the desired trait. HTS at NREL is currently focused on the following problems: screening for improved enzymes generated by molecular evolution-type protocols, assaying cellulase preparations by automated filter paper assays, and obtaining a high-capacity liquid handling/plate handling system for doing higher throughput of randomly altered enzymes.

Cellobiohydrolases (CBH I) Expression

To date, *T. reesei* CBH I is the most effective cellulase component known. The performance of this enzyme would be enhanced, however, if its use at elevated temperatures were possible. Protein engineering principles cannot be used to improve this enzyme until effective hosts for the *cbh1* gene are found. This work is a continuing effort.

Milestone Progress/Completion

C Milestone # 232 – "Investigate and develop heterologous expression systems suitable for cellobiohydrolases" (9/30/01). The objective of this milestone was to investigate heterologous host expression systems suitable for the expression of cellobiohydrolases, specifically for use in HTS applications. This objective was met for the *E. coli* host and a milestone report was completed on schedule.

Requirements for HTS include the absolute requirement that the gene encoding the cellobiohydrolase be functionally expressed in a suitable microbial host, and that there is an effective screening procedure for the desired activity of improved function. Because of this requirement, the choice of an expression system for the high-level production of recombinant cellulases depends on many host factors that are essential for the development of directed evolution technologies. These include cell growth characteristics, expression levels, intracellular and extracellular expression, posttranslational modifications, and ultimately the biological activity of the protein of interest. The many advantages of *E. coli* ensure that it remains a valuable organism for the high-level production of recombinant proteins including cellulases and is generally the preferred host for HTS applications. However, in spite of the extensive knowledge of

the genetics and molecular biology of *E. coli*, it is clear that not every gene can be expressed effectively in this organism.

P Milestone # 263 – "Acquire gene coding alternative fungal CBH I and determine its DNA sequence" (6/30/01). Efforts to isolate genes for non-*Trichoderma reesei* CBH I enzymes have not been successful this year. Genomic DNA was collected from five strains of filamentous fungi. Using BLAST™ search routines, several highly conserved amino acid sequence motifs were identified for the glycosyl hydrolase family 7 cellobiohydrolases. This sequence information was then used to design primers to probe genomic DNA collections. So far, only the gene coding CBH I in *T. reesei(viride)* has been positively identified. We are now redesigning both sequence motifs and primers for better specificity. We wish to continue this work under a new P milestone next year.

P Milestone # 264 – "Acquire gene coding *Aspergillus* sp. CBH I and clone into a new host organism for expression," (6/30/01). This milestone was deleted because since we have not been able to isolate the genes coding other CBH I enzymes this year (see P Milestoen # 263 above), the objective to clone them into *A. awamori* was not met. In addition, the essential strategy underlying this milestone is now in question. That is, we wanted to use one *Aspergillus* strain known for naturally low levels of cellulase production as a host for *cbh1* genes from another strain of *Aspergillus* (which does produce cellulases). In this case, we would expect the target *cbh1* genes to be readily expressed in active form from the noncellulase producing *Aspergillus*. This system would allow us to test site-specific mutations in CBH I by expressing modified genes in an *Aspergillus* host without its own CBH I to complicate purification.

Although the general approach is still meritorious, we have not been able to confirm that *A. aculeatus* produces CBH I enzymes in high titer and have encountered great difficulty purifying CBH I from induced cultures of *A. aculeatus* at NREL. This contrasts from the literature, mostly Japanese, where this strain of *Aspergillus* is reported to produce large quantities of CBH I. We now feel that either these reports were in error or the strains used in Japan differ from those we acquired from ATCC.

P Milestone # 265 – "Assess utility of *E. coli* variant strains for expression of CBH I and prepare manuscript" (8/30/01). This P milestone was deleted because the objective is identical to C Milestone # FY01-232 "Investigate and Develop Heterologous Expression System(s) Suitable for Cellobiohydrolases" described above.

Progress Highlights and Issues

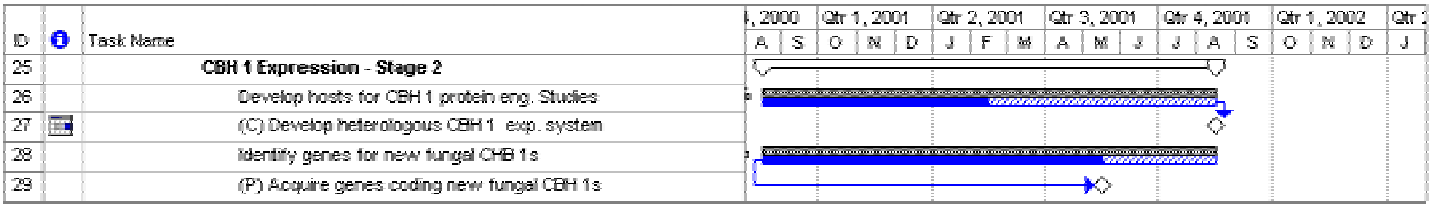

Figure 15: Cellobiohydrolase I Expression. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01

Cellobiohydrolases, including CBH I from the mesophilic mold *Trichoderma reesei*, are major contributors to hydrolysis of microcrystalline cellulose. To order to enzymatically hydrolyze biomass more effectively to its component sugars; it is essential that key improved enzymes are either developed or discovered. This year we investigated in great detail *E. coli* as a potential heterologous expression system for cellobiohydrolases. We have been able to express large quantities of soluble *T. reesei* CBH I catalytic domain (CD) in *E. coli* using maltose binding protein fusions and have purified the recombinant protein to homogeneity using affinity, anion exchange, and SEC. An evaluation of the purified fusion protein showed no detectable activity on either soluble synthetic substrates such as *p*NP-β-D-lactopyranoside or on amorphous cellulose (phosphoric acid swollen cellulose). This finding suggests that the conformation of *E. coli* expressed CBH I is incorrect (non-native), thus limiting the usefulness of this host for future high throughput applications.

Subcontracts

J. Sakon, University of Arkansas
XDH-0-30009-02
XDH-0-30009-02 mods 1, 2, and 3
Provide Support for Cellulase Engineering
4/04/00-9/30/01
The goals of this subcontract include structure determinations of enzymes vital to the project objective. These data are also key for planning protein-engineering efforts. For the past three years, the subcontractor has successfully crystallized and solved the structures of *Acidothermus cellulyticus* E1cd, E1cd in complex with cellotetraose, and every mutant that NREL provided. Careful analysis of these structures has clarified the enzyme mechanism of *A. cellulolyticus* E1 and has helped us design a catalytically enhanced mutant. The subcontractor recently contributed to the design of surface mutations of EIcd which were used to probe its interaction with cellulose. Immediate goals also include analysis of *T. reesei* CBH I and identifying strategies to improve its thermal stability.

Enzyme Subcontract Liaison

The liaison is working to ensure good communication and planning between NREL and major cellulase subcontractors (Genencor International and Novozymes), coordinating sample testing and data transfer, and planning out-year activities with subcontractors.

Milestone Progress/Completion

K Milestone # 247 – "Genencor Subcontract – 2X Cost Improvement in Cellulase Enzyme" (8/30/01). Monthly subcontract research meetings between NREL and GCI scientists have clarified the Cellulase Cost Metric (CCM) and these findings were reported in a P milestone "Establish Clear Statement of Deliverable for GCI K Milestone." We now understand the origins of cellulase cost at commercial scale well enough to proclaim "technologies conferring a 2X cellulase cost reduction (relative to existing industrial practices) have been validated."

P Milestone # 319 – "Establish clear statement of deliverable for K Milestone," (2/28/01). This objective was completed on 4/24/01. For cellulase enzyme to be commercialized in

a biomass-to-ethanol process, the conversion cost must be reduced. To measure enzyme cost reduction, a metric has been developed that incorporates technical and economic parameters of enzyme production. This metric shows that the cost of enzyme in a biomass-to-ethanol process is dependent on enzyme price, enzyme loading, enzyme concentration, and process ethanol yield. To calculate enzyme cost in $ per gallon ethanol (C_E), the enzyme price ($/L product, E_P) is required from the enzyme producer, along with enzyme product samples. From these samples, the enzyme loading (g protein/g cellulose entering hydrolysis, E_L) is determined by DSA or SSF for the supplied enzyme concentration (g protein/L product, B_N), which is measured through desalting HPLC. Ethanol yield (gallon ethanol/g cellulose entering hydrolysis) is dependent on the ethanol process design.

P Milestone # 318 – "Conduct Bi-monthly research meetings with GCI" (8/30/01). This objective was met as planned. Bi-monthly research update meetings between Genencor and NREL scientists were conducted on September 29, 2000 (NREL); January 25, 2001 (NREL); April 27, 2001 (Palo Alto, CA); May 26, 2001 (NREL); June 6, 2001 (Washington, DC); and August 2, 2001 (Proctor, NH).

Progress Highlights and Issues

The GCI subcontract is monitored via bi-monthly meetings and reports. The Novozymes subcontract will be managed in a similar fashion. The NREL staff serving a subcontract monitors (Himmel and Nieves) are also coordinating testing and benchmarking activities to ensure efficient and equivalent support for both subcontractors.

Non-Cellulase Enzymes

We will monitor a university subcontract studying lignin depolymerases (LD) and begin planning activities for the future NREL research project targeting lignin-degrading enzymes.

Milestone Progress/Completion

P Milestone # 266 – "Place subcontract with the University of Minnesota" (1/30/01). The subcontract was placed on 5/15/01.

Progress Highlights and Issues

In FY 01, the NREL Director' Discretionary Research and Development Fund (DDRD) project "Validation of the First Lignin Depolymerase" supported the preliminary stages of LD investigation carried out by NREL researchers thereby leveraging the Biofuels funding of the subcontract.

Subcontracts

S. Sarkanen, University of Minnesota
XCO-1-31048-01
"Lignin Depolymerase from *T. cingulata*"
5/15/01-5/14/02
It had been widely felt that lignin peroxidase, manganese-dependent peroxidase, and lactase are the primary agents responsible for depolymerizing lignins *in vivo*. However, these enzymes can both polymerize and depolymerize lignins; none of them have been

reported to bring about complete (or even 50%) depolymerization of a polymeric lignin sample *in vitro*. It is therefore unlikely that they could be responsible for governing the first arduous step in the lignin biodegradative pathway. However, an enzyme has been isolated at the University of Minnesota from *Trametes cingulata* that completely depolymerizes high molecular weight lignin components. One objective of this work involves characterizing the lignin depolymerase enzyme and producing the active recombinant protein in a suitable host.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

- M. Himmel served as Vice-Chair of the Gordon Research Conference on "Cellulases and Cellulosomes" held in Proctor, NH July, 2001.

- General presentations at NREL to Genencor, Novozymes, and numerous academic and congressional tour groups.

- S. Decker gave numerous presentations of the HTS facility to academic, DOE, and USDA visitors.

- M. Himmel and T. Vinzant attended a course on MALLS held at Wyatt Engineering in Santa Barbara, CA in July.

- J. Baker attended a training course in InsightII at the Accelrys Training center in San Diego, CA.

- S. McCarter gave a presentation on the New Idea Project "Flow Cytometry" to the NREL NBC staff.

Scientific Meetings: Papers/Posters Presented or Recently Accepted for Presentation

- "Cellulase/cellulose interaction: Site directed surface modifications of a family 5 endoglucanase." S. L. McCarter, W. S. Adney, T. B. Vinzant, F. Posey-Eddy, J. O. Baker, . Rignall, J. Sakon, and M. E. Himmel, SIM Annual Meeting, July 25-30, 2001.

- "Diversity and Conservation of Relevant GH Families in Well-Studied Cellulolytic Microbes," S.-Y. Ding, W.S. Adney, S.M. McCarter, S.R. Decker, T.B. Vinzant, and M.E. Himmel. Gordon Research Conference on "Cellulases and Cellulosomes," Poster A3, Proctor Academy, New Hampshire, July 25-Aug 3, 2001.

- "Temperature and Enzyme Loadings as Variables in Cellulase Kinetics," J.O. Baker, T. R. Rignall, W. S. Adney, S. L. McCarter, T. B. Vinzant, S. R. Decker, and M. E. Himmel. Gordon Research Conference on "Cellulases and Cellulosomes," Proctor Academy, New Hampshire, July 25-Aug 3, 2001.

- "Enzymatic Depolymerization of Biomass Carbohydrates: New Perspective", M.E. Himmel, Fifth Biomass Conference of the Americas, Orlando, FL, September 17-21, 2001.

Scientific Journals: Papers accepted for publication

- "Cellulase Animation," M. Himmel and D. Seely, Run time 11 min., NREL, Golden, CO. 2000. Copyright DOE/MRI PAU2-568-354.

- "Fingerprinting *Trichoderma reesei* Hydrolases in a Commercial Cellulase Preparation," T.B. Vinzant, W.S. Adney, S.R. Decker, J.O. Baker, M.T. Kinter, N.E. Sherman, J.W. Fox, and M.E. Himmel, Appl. Biochem. Biotechnol. 99-107 (2001).

- "Exploration of the Cellulose Surface Binding Properties of A. *cellulolyticus* Cel5A by Site Specific Mutagenesis," S.L. McCarter, W.S. Adney, T.B. Vinzant, F. Posey-Eddy, S.R. Decker, J.O. Baker, J. Sakon, and M.E. Himmel, Appl. Biochem. Biotechnol. 2002, In press.

- "Effect of a Single Active-Site-Cleft Mutation upon Product Specificity in a Thermostable Bacterial Cellulase," T.R. Rignall, J.O. Baker, S.L. McCarter, W.S. Adney, T.B. Vinzant, S.R. Decker and M.E. Himmel, Appl. Bioachem. Biotechnol. 2002, In press.

STRATEGIC PROJECTS

Summary of Technical Achievements or Results

As a result of the Gate 2 Review of the original Corn Fiber Project held on 12/14/01 and subsequent discussions with ethanol industry stakeholders and USDA researchers involved in corn ethanol research, the project evolved into three separate projects. These projects are:

- A one-year CRADA with Broin and Associates to improve dry mill ethanol plant operations.
- A project to investigate converting Distillers Grains (DG) to ethanol and high-value feed product for the swine and poultry markets.
- A project in collaboration with USDA and the University of Illinois to investigate the development of a process for converting "Quick Fiber" to ethanol.

General Technical/Scientific Progress

Milestone Progress/Completion

P Milestone #327 – "Preliminary Options for Improving the Process Economics of Corn Dry Mills" (9/30/01). This milestone no longer reflects the work in progress and so was not completed as originally planned in FY01. In its place new milestones for the three separate projects related to corn mills will be developed for FY02.

Broin CRADA

Milestone Progress/Completion

No milestones were planned for this performance period (due to the delay in signing the CRADA).

Progress Highlights and Issues

Preliminary evaluation of the process and economic impact of options for improving water management is under way. Promising results were obtained through bench scale experiments and limited pilot equipment testing. Further testing of various pilot plant equipment is required to determine their performance. NREL will review the experimental and process simulation results with Broin before making recommendations for the next development activities.

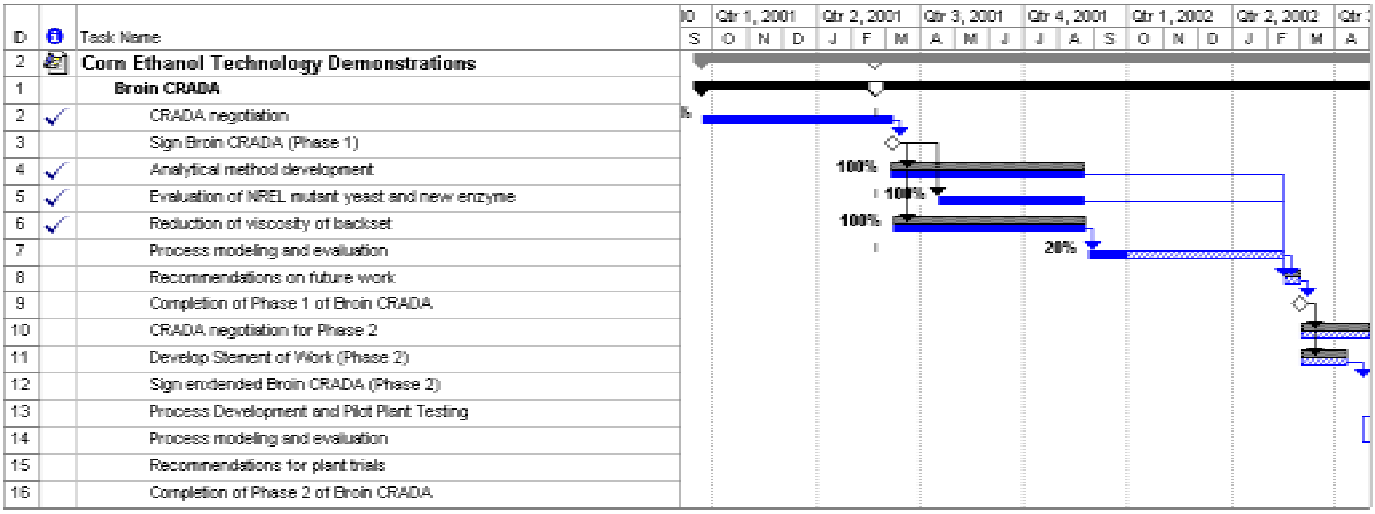

Figure 116: Broin CRADA. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01.

DDG Conversion Project

Milestone Progress/Completion

No milestones were planned for this performance period.

Progress Highlights and Issues

Discussion with potential industrial partners on collaboration in distillers grains conversion research and development is currently in progress. Richard Eichstadt of the Midwest DDGS Association was contacted to assist in collecting wet grain samples from several dry corn mills for analysis of composition and potential ethanol yield.

Steam pretreatment (without added acid) of brewery wet spent grain solubilizes approximately 40% of the input material. A series of screening SSF experiment was carried out to assess the performance of several commercial enzymes (cellulase, glucoamylase, and xylanase). The samples are being analyzed to determine the conversion yields.

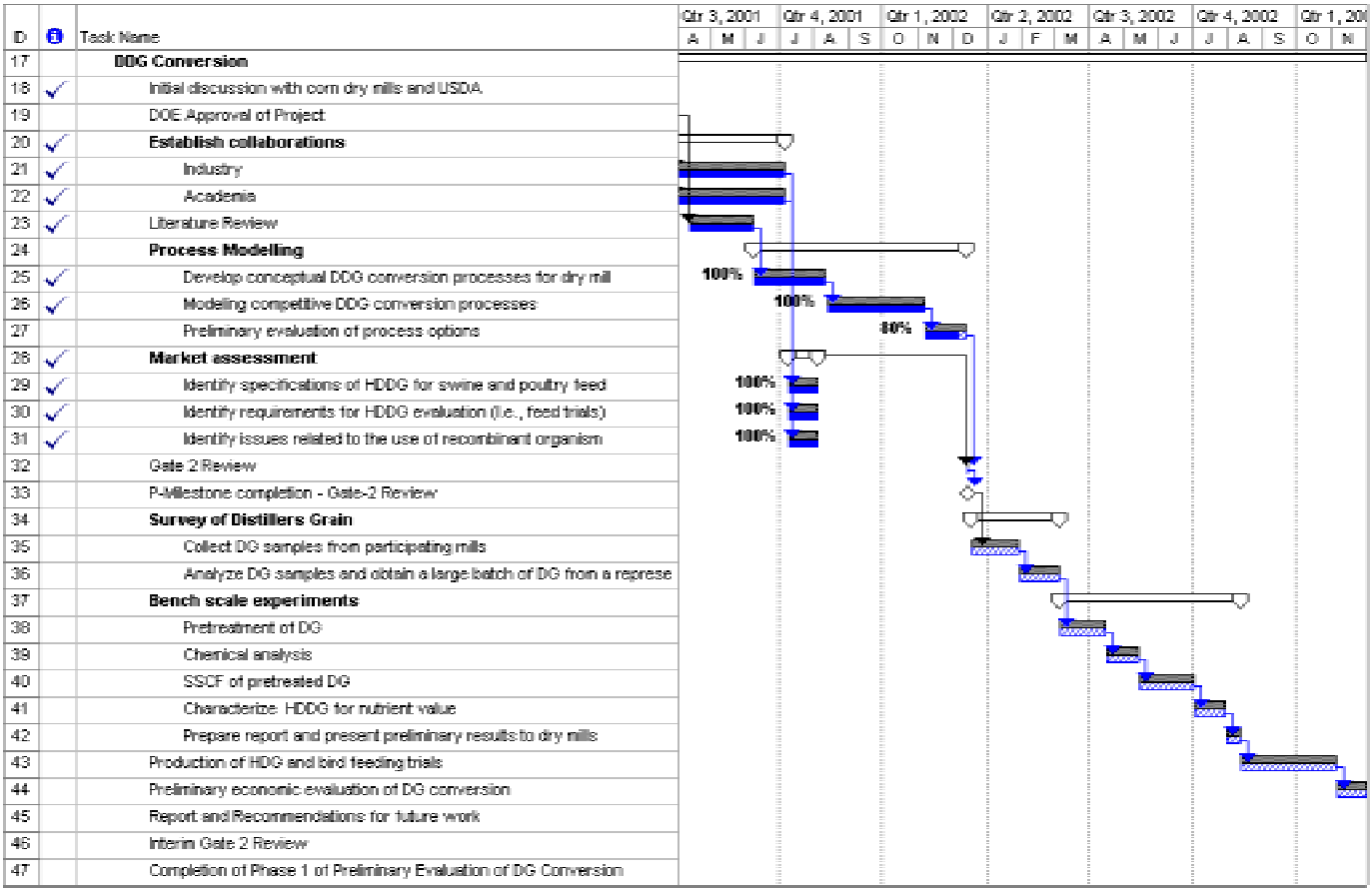

Figure 127: DDG Conversion Project. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01.

Advanced Corn Mills Project

Milestone Progress/Completion

No milestones were planned for this performance period.

Progress Highlights and Issues

Collaboration between NREL, USDA/ARS ERRC (Wyndmoor, PA), USDA/ARS NCAUR (Peoria, IL) and the University of Illinois on the development of a process for converting Quick Fiber to ethanol is under way. Quick Fiber is de-germed fiber obtained from a hybrid wet/dry corn milling ethanol process developed by the University of Illinois. A joint statement of work is being developed. Key research activities include: (1) Quick Fiber Production (University of Illinois), (2) Pretreatment of Quick Fiber (NREL), (3) Enzymatic hydrolysis and ethanol fermentation of pretreated fiber (NCAUR), and (4) Process economic evaluation (ERRC and NREL).

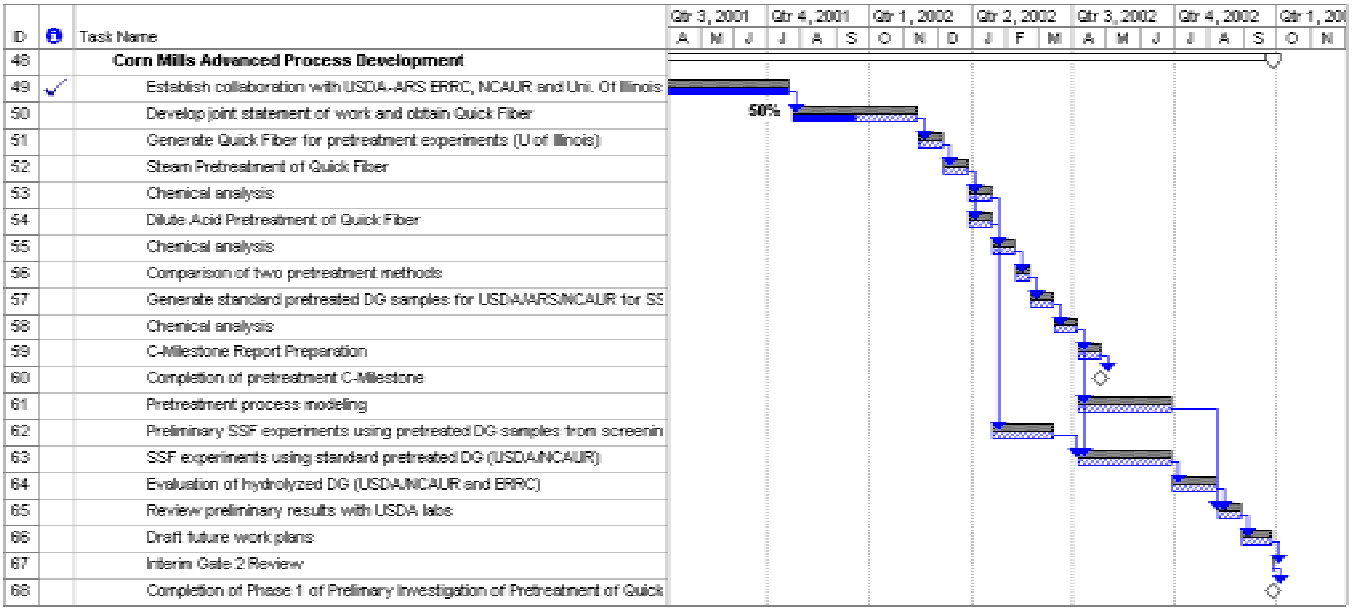

Figure 138: Advanced Corn Mills Project. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

- Bob Wooley, Quang Nguyen and Kely Ibsen presented an overview of the Distillers Grains Conversion Project to an invited audience (dry corn ethanol plant managers, DDGS brokers, USDA/ARS staff, and academia) at the 17th International Fuel Ethanol Workshop in St. Paul, MN.

- Quang Nguyen and Kelly Ibsen visited the USDA/ARS ERRC (Wyndmoor, PA) and discussed with ERRC technical staff regarding areas for collaboration in the development of advanced dry corn mill ethanol technologies.

LIGNIN CONVERSION TO FUELS

Summary of Technical Achievements or Results

This project is currently working towards completing Stage 2 of the development of a process for converting lignin to a high-octane hydrocarbon product. Research is focused on further optimizing the three main steps in the process: lignin extraction, depolymerization, and hydroprocessing. A Gate 3 review took place in July 2001 fulfilling a C-level milestone for the project. At the review, we decided to continue Stage 2 work because sufficient progress had not been made towards meeting all of the performance targets set in the Gate 2 review in October 2000. The review identified six areas where significant work needs to be done before the project can move to Stage 3. The areas are:

1. Regulatory issues

2. Effects of the lignin product on fuel properties

3. Market issues

4. Interactions between the lignin and ethanol processes

5. Process issues

6. Alternative chemical products from the BCD process

Research in Stage 3 will be aimed at further developing and scaling-up the three main steps of the process leading to integration of the process by the end of 2003.

A P-level milestone "Conduct Performance Verification of University of Utah's Operation for Making a Lignin-Derived Hydrocarbon Fuel Product" was also completed during the reporting period. The purpose of this milestone was to verify performance of the Lignin Conversion to Fuels process operated at the University of Utah. The milestone was successfully completed by NREL researchers going to the University of Utah and witnessing all stages of the process being performed and verifying reaction yields at each stage.

Samples were collected from the various stages for subsequent characterization at NREL. The performance of one base catalyzed depolymerization (BCD) run and two hydroprocessing (HPR) runs performed in the flow reactors at the U. of Utah were witnessed. Operationally, the BCD run was problem-free and the mass closure (<98%) on the run was very good. The yield of the phenolic intermediate (BCD product) for use in the next step of the process, hydroprocessing, was about 62%, which was lower than the target of 75%. Gas, mostly carbon dioxide, was a significant product (yield 14%) from this run as well as the insoluble residue (yield 21%). Two HPR runs were also performed successfully; however, the overall yields (25% and 38%, respectively, based

on the amount of BCD product fed) of product in the gasoline boiling range from the runs were lower than expected. Combining the yield from the BCD run with the yield of hydrocarbon product from the second HPR run gives a verified yield of hydrocarbon product that could be blended with gasoline of about 25% based on the lignin feed.

This is half of the target yield that has been used in process economic calculations. Work continues to improve yields from both the BCD and HPR processes.

General Technical or Scientific Progress

Milestone Progress/Completion

P Milestone # 321 - "Initial Assessment of Hydrocarbon Product Generated in Flow Reactors for Both the BCD and HPR Steps" (1/31/01). The milestone was completed on schedule.

P Milestone # 322 – "Conduct Performance Verification of University of Utah's Operation for Making a Lignin-Derived Hydrocarbon Fuel Product" (4/30/01, delayed to 6/30/01). Completed per the revised schedule.

C Milestone #235 – "Conduct Gate 3 Review" (6/30/01, delayed to 7/31/01). Completed per the revised schedule.

P Milestone #323 – "Complete Report Summarizing all Investigations into the Base Catalyzed Depolymerization Reaction Chemistry of Lignin and Lignin Model Compounds" (9/30/01). Work is proceeding and the milestone will be completed in 12/01.

Progress Highlights and Issues

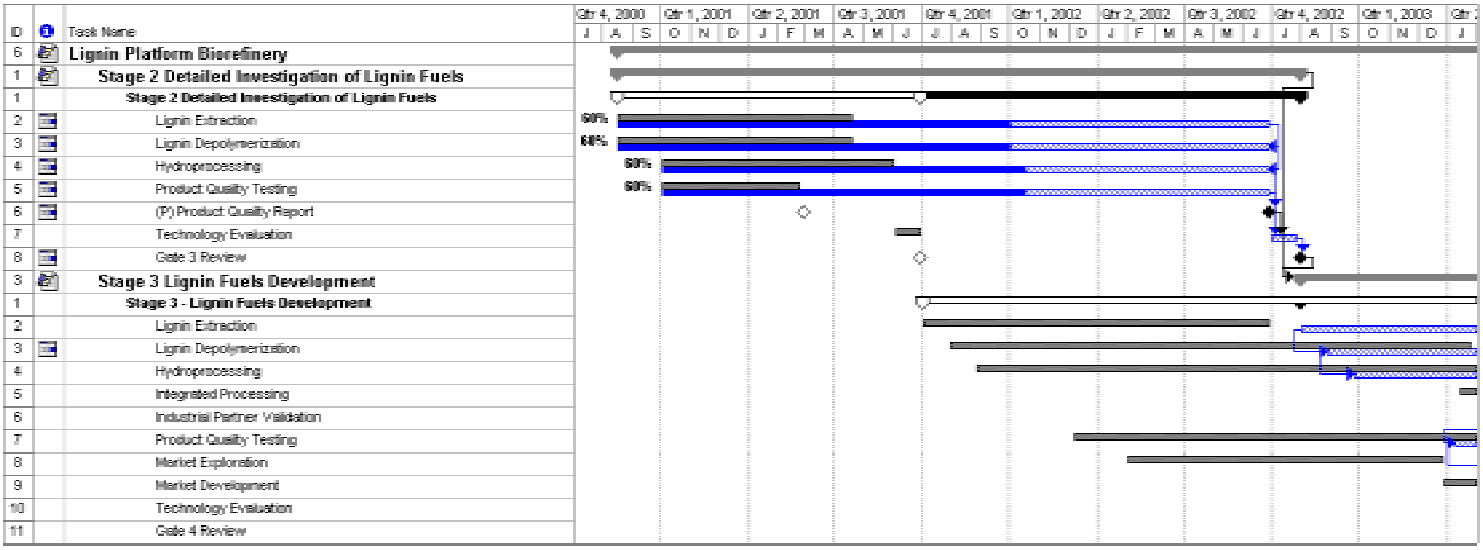

Figure 149; Lignin Platform. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01

Lignin Extraction

No further work has been done on the extraction of lignin from SSF residues made from Yellow Poplar or other biomass feedstocks. Completion of the lignin extraction task cannot occur until samples of SSF residue made from corn stover are obtained. We expect a corn stover derived SSF residue to be supplied by the Bioprocess Development Team. Tests of the lignin extraction process will then be completed with this residue.

A purchase order has been placed with the University of Sherbrooke, to buy 30kg of lignin made from hardwoods to supply the subcontractors at the University of Utah with material for their research. Half of the lignin came from their supply of Alcell lignin, which was made by Repap Technologies Inc. by an organosolv process from mixed hardwoods. This is the same lignin as has been used in the majority of the work performed at the University of Utah. The second 15kg of lignin will come from steam exploded aspen. The lignin is extracted from the steam-exploded lignocellulose with aqueous alkali.

Lignin Depolymerization and Hydroprocessing

The P-level milestone "Conduct Performance Verification of U. of Utah's Operation for Making a Lignin-Derived Hydrocarbon Fuel Product," completed during the reporting period assessed the status of the process relative to the performance targets needed for the Lignin Fuels project to move to the Developmental Stage. The milestone was successfully completed by David Johnson and Esteban Chornet going to the University of Utah during the period from June 11-15, 2001, and witnessing the performance of one base catalyzed depolymerization (BCD) run and two hydroprocessing (HPR) runs performed in their flow reactors. Operationally, the BCD run was problem-free and 0.5 kg of lignin were processed at the desired reaction conditions. The work-up of the product was observed and this also went as planned. The mass closure on this run was very good with about 98% of the lignin fed appearing in the products collected. The yield of the phenolic intermediate (BCD product) for use in the next step of the process, hydroprocessing, was about 62%. Gas, mostly carbon dioxide, was another significant product (yield 14%) from this run as was the insoluble residue (yield 21%). The yield of gas was higher than had been observed by the Utah researchers in previous runs and is the most likely cause of the below target (75%) yield of BCD product.

Prior to this run the Utah researchers had changed the packing in the BCD reactor resulting in an increase in the residence time. This could be the cause of the higher gas yield. The HPR runs were also operationally successful with about 100g and 30g of BCD product processed in the two runs, respectively. The two runs also used different feed rates of 50 and 25 g/h. The HPR products were distilled at atmospheric pressure and large fractions of the products were found to distill within the gasoline boiling range (<195 °C). The overall yields of product in the gasoline boiling range were, however, lower than expected (25% and 38%, respectively, based on the amount of BCD product fed). Combining the yield from the BCD run with the yield of hydrocarbon product from the second HPR run gives a verified yield of hydrocarbon product that could be blended with gasoline of about 25% based on the lignin feed. This is half of the yield that was set as a target for the project to progress to the Developmental Stage. Work continues to improve yields from both the BCD and HPR processes. Fifteen samples were collected

from the three runs performed during the visit. The samples have been sent to NREL for chemical analysis so we can assess product quality.
The chemistry of base catalyzed lignin depolymerization continues to be studied at NREL. Using model compounds it has been shown that the compounds that can form from depolymerization of lignin, such as catechol, guaiacol, vanillin, vanillic acid, vanillyl alcohol, and syringol, undergo polymerization to a small extent in addition to the previously known reactions of decarboxylation, decarbonylation, demethylation, methylation, and demethoxylation. With all of the reactions, increasing reaction severity was seen to have a greater effect than base concentration on the extent to which they occur. Carbon dioxide appeared to form to only a limited extent in compounds that do not contain a carboxylic acid function. Polymerization occurred to the greatest extent when an alcohol group was present in the side chain. From the UV spectra of the polymeric products it appears that they are more chromophoric than the starting compounds, possibly due to them having a quinone-type structure. Future work will further examine the chemical nature of the polymeric products so that the mechanism of their formation can be understood and their formation can be minimized.

Product Quality Testing

This project has become involved in an effort to develop internationally accepted standard methods and reference materials for lignin analysis. In the past month we have been involved in a round robin on the use of Fourier transform infra-red spectroscopy (FTIR) of lignins. Twenty laboratories from around the world have committed to analyze two lignins using an accepted protocol as well as their own protocols. The main goal of the round robin is to assess the reproducibility of mid-range FTIR spectra of lignin in different laboratories throughout the world using the same protocol. Another goal is to assess how the spectra are changed by changing the spectroscopic equipment, the sample presentation, and the mathematical treatment of the spectral data. FTIR spectra are often used to quantify the functional groups in the lignin structure. The round robin will also assess how different laboratories extract this data from the spectra of the two lignins.

One of the recommendations from the Gate 3 review was that more emphasis be placed on evaluating the affect of the lignin-derived blending agent on emissions from the blended fuel and the performance of the blended fuel rather than octane improvement. The reviewers felt that engine testing with the fuel was not necessary at this stage but that it was more important to measure the fuel properties that are used in assessing toxic emissions and the Driveability Index. Toxic emissions are assessed using the EPA's Complex Model spreadsheet, which was sent to us by Dr. J Hadder (ORNL) who was one of the attendees of the review. The inputs to the Driveability Index can be taken from the distillation curve of the fuel. These inputs are the values of T_{10}, T_{50}, and T_{90}, the temperatures at which 10%, 50% and 90% of the fuel are evaporated. The Complex Model spreadsheet requires knowledge of the amounts of any oxygenates that are present in the fuel, the sulfur content of the fuel, the contents (volume %) of benzene, aromatics and olefins in the fuel, and the volatility of the fuel based on its Reid Vapor Pressure, E200 and E300 parameters. E200 and E300 are also obtained from the distillation curve of a fuel and are the amounts of the fuel that are evaporated at 200^{o}F and 300^{o}F. Using the spreadsheet it is simple to evaluate the affect of changing fuel properties on the expected emissions from a fuel. For example decreasing sulfur content from the base

level (339 ppm) to 30 ppm has the largest affect on benzene emissions, which are reduced by almost 12%, then NOx and Total Toxics, with only a small affect on Total VOCs. Future product quality testing will focus on conducting the tests necessary for the Complex Model and the Driveability Index. One consequence of this is that we will not need a liter of fuel for the tests but will be able to conduct the tests with only about 0.2 liters of fuel.

Subcontractors

J. Shabtai, University of Utah
XCG-9-29011-01
Continued Studies into the Conversion of Lignin into High-Octane Aromatic Hydrocarbon Fuel Additives
10/1/00 - 9/30/01
Progress is described in the section on lignin depolymerization and hydroprocessing.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

- Esteban Chornet and David Johnson traveled to Salt Lake City on May 9-10, 2001, to conduct a performance verification of the University of Utah's operation for making a lignin-derived hydrocarbon fuel product." This visit was shortened because of operational problems with the BCD flow reactor. The visit in June was then planned to be longer so that verification of the process could be completed.

- Esteban Chornet and David Johnson traveled to Salt Lake City on June 11-15, 2001, to conduct a performance verification of the University of Utah's operation for making a lignin-derived hydrocarbon fuel product."

Scientific Meetings: Papers/Posters Presented or Recently Accepted for Presentation

- A special session on "Lignin – An Untapped Resource" was held on the afternoon of May 7 at the 23rd Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, CO. The special session was organized by members of the Lignin Conversion to Fuels Project. The presentations made included:

    - "An Overview on Lignin, Current Research Trends and Industrial Applications" by Jairo Lora, Granit S. A.

    - "A Case Study on the Industrial Production of Lignin and Lignin-derived Products in the Pulp & Paper Industry" by Bob Benson, Tembec Inc.

    - "The Hydrolytic Depolymerization of Lignin as a Route to Oxy-aromatics," by Esteban Chornet, NREL

    - "Alkyl-aromatic Octane Enhancers from Lignin" by Joseph Shabtai, University of Utah

- "Physical and Chemical Characterization of Lignin and Depolymerized Lignin Products" by David Johnson, NREL

- "Hydrogenolysis of Lignins: Influence of the Pretreatment Using Microwave and Ultrasound Irradiations" by Adilson R. Gonçalves, Department of Biotechnology-FAENQUIL, Brazil

Scientific Journals: Papers Accepted for Publication

None

INDUSTRIAL PARTNERSHIPS

Summary of Technical Achievements or Results

Employing NREL's expertise in genetic engineering, milestones for the first year of the Cargill-Dow CRADA entitled "Cargill-Dow Lactic Acid and Ethanol Bioenergy Project" were completed on time. The utilization of sugars from biomass for the production of high value products will help achieve the goal of tripling the nation's production and use of biobased products and bioenergy. These accomplishments will advance Cargill-Dow's ability to utilize its proprietary tools to achieve their share of this goal.

The Industrial Partnership Team attended and made presentations at five ethanol workshops sponsored by the DOE Regional Biomass Energy Program (RBEP). The workshops were held in Nebraska, Alaska, Minnesota, Pennsylvania, and Puerto Rico and were well attended with over 100 participants at most of the meetings. The emphasis in NREL's presentations was describing ongoing work on the Enzyme Sugar-Ethanol Platform, and the tie-in with the existing corn ethanol industry. The participants were quite interested in learning about lignocellulosics- to-ethanol technology, particularly in Minnesota, Alaska, and Nebraska. Follow-up meetings with parties in Minnesota have led to collaborations on corn dry mill improvement projects (see the Strategic Projects section).

Under the Collins-Pine subcontract several tons of softwood thinnings from the Collins-Pine area were shipped to Jennings, Louisiana for lignin production. This lignin will be used to perform large-scale testing this coming fiscal year at the Chester biomass power facility.

TSS identified an opportunity for Department of Energy (DOE) and National Renewable Energy Laboratory (NREL) to jointly fund projects that will support the creation of a new biomass to ethanol industry in California. The passage of new California legislation that provides significant annual funding for renewable electrical power plants is available to co-fund research for co-located biomass to ethanol plants with NREL/DOE. The Industrial Partnerships team continues to search for ways to take advantage of California's large biomass resources and need for ethanol by supporting the lignocellulsoics-to-ethanol efforts in California.

General Technical or Scientific Progress

Industrial Partnerships

Milestone Progress/Completion

C Milestone # 234 – "Complete evaluation of bioethanol co-location with a coal power station in the Midwest U.S." (7/30/01).
Proposals for the subcontract to accomplish this work and milestone have been received and are being reviewed. The milestone due date has been moved to 5/31/02.

Subcontractors

J. Cherry, Novozymes Biotech, Inc.
ZCO- 1-30017-02
Cellulase Cost Reduction for Bioethanol
12/19/00 -12/19/03
Novozymes Biotech Inc recently conducted its first review under the "Cellulase Cost Reduction" subcontract. The review punctuated the proprietary strains, enzymes, and techniques that Novozymes is bringing to bear on this project. Research updates include the acquisition and setup of all new equipment, cloning of new cellulase genes from microorganisms, expression of cellulases in surrogate hosts for further manipulation, and improved methods for analysis of secreted proteins from cellulolytic organisms. They continue to identify and clone novel genes from microorganisms and are now concentrating on the expression and characterization of a selected number of them. The first genomic DNA library for use in gene expression analysis has been constructed and a compilation of unique expressed sequence tags (ESTs) has been assembled. Analysis of new strains grown on selected feedstocks suggests that there are many different proteins produced when compared to normal fermentation conditions." The next review meeting will be in Davis, California, on October 23, 2001.

N. Hinman, BC International (BCI)
ZCO-0-30019-01
Gridley Ethanol Project Development
11/30/00 - 5/31/02
A no-cost extension was granted to BC International until May 31, 2002. Numerous task deadlines were extended to assure completion. BCI-Gridley, LLC was legally formed and members of the LLC have been established. Agreements are in place which define the rights and obligations of the members. The Rice Straw Coop has been legally formed; it's functioning and feedstock supply agreements are in place. The Rice Straw Coop continues to bale rice straw to further test and evaluate equipment and operations. This fall, 18,000 bone dry tons of rice straw is being collected and stored on a 25-acre lot. This is the amount of straw needed for the "Demonstration Scale" of the Gridley project. Recently, the Coop was awarded a grant from CARB to support the baling, road siding, trucking, and storage tests and operations. The plan is to bale 100,000 dry tons in the fall 2002. Additionally, extensive compositional tests were completed on different varieties of rice straw as a function of soil type and harvest operations.

Validation testing has been done on the 1st and 2nd stage hydrolysis on a pilot scale, and fermentation on a bench scale. Extensive tests have been performed on rice straw lignin to examine the potential for slagging, fouling, deposition, corrosion, emissions and ash distribution. Additionally, extensive tests have been performed on options to remove silica.

N. Hinman, BC International (BCI)
XCO-0-29068-01
Plumas County Ethanol Project
11/30/00 - 5/30/02

The goal of this subcontract is to demonstrate the suitability of lignin residues from ethanol production for use as a boiler fuel in the Collins-Pine boiler at the Chester, California site. The feedstock supply study was completed and several tons of softwood thinnings from the Collins-Pine area were shipped to Jennings, Louisiana for lignin production. Lignin production was initiated at a rate of production of 700-800 lbs. per day. Softwood lignin is being tested for potential slagging, fouling, deposition, corrosion, emissions, and ash disposition at the bench scale. The next step will be to perform a large-scale burn test at EERC. Before proceeding, bench scale lignin sample composition will be compared with the large-scale lignin (ton quantities) that is being produced for the large scale test run to assure continuity of composition.

L. Forrest, TSS Consultants
YDH -0-30010-01
Logistics Support and Development of Biomass Energy in the Western United States
2/7/00 - 2/6/02
The denial of the oxygenate waiver to the state of California will require this state to either import or produce the ethanol required to meet the EPA emissions standards. This EPA decision, essentially doubles the demand for ethanol in California from 300 to 600 million gallons annually. TSS continued to provide reports and data to private sector stakeholders that are interested in creating a new commercial biomass-to-ethanol industry in the west. These stakeholders included farmer's groups and associations, such as the Farm Bureau; winery and sugar beet growers associations; rice growers associations; owners of existing and proposed new biomass power plant associations; timberland and sawmill owners generating biomass wastes, and waste industry haulers; landfill managers; and transfer station operators. In addition, TSS worked with state and local government officials, including rural county supervisors, Resource Conservation Districts, utility and power plant regulators by providing information from DOE Studies and NREL reports. These reports demonstrated the feasibility and key issues or barriers to creating a new biomass to ethanol industry that would use biomass wastes from forests, urban waste streams and agricultural residues.

TSS identified an opportunity for DOE and NREL to jointly fund projects that will support the creation of a new biomass to ethanol industry in California. This was basically the passage of new California State Legislation that provides significant annual funding for renewable electrical power plants. This funding is available to co-fund research for co-located biomass to ethanol plants with NREL/DOE. Co-location offers a potentially promising scenario in California as there are a number of operating biomass power plants in the state. There are opportunities to co-locate ethanol facilities as part of an existing or new proposed electrical power plant and the CEC has indicated an interest in jointly funding these projects with DOE/NREL during the coming year.

M. Smith, MSS Consultants
YDH -0-30010-02
Logistics Support and Development of a Bioethanol Market in the Western U.S.
2/3/00 - 2/2/02
MSS Consultants served NREL as a liaison with state and local agencies, and private organizations with regard to biomass energy issues and reported its findings to NREL

technical monitor in its monthly reports. Due to financial shortfalls for sustaining the executive director of the Western Biomass Consortium, no meetings were held in the last six months.
Due to the government's phase out program of MTBE, North Eastern states as well as California are more open to ethanol as a fuel additive. An avenue open for possible advancement is the oil companies. Refineries such as Shell, BP, Amoco and Marathon are interested in "greening" themselves in the future.

MSS Consultants met with a variety of business executives that are involved in ethanol or biomass related activities, including: U.S. Departments of Agriculture, Forestry and Interior, Iogen Corporation, BP Amoco, Biotechnology Industry Organization, New Uses Council, National Farmers' Union, Clean Fuels Development Coalition, California Energy Commission, Genencor, Sealaska Native Corporation, the Denali Commission, Renewable Fuels Association, Northwest Natural Resource Group, National Association of State Foresters, Oregon State Energy Office, Public Power Institute, Wildland Fire Coordinator, U.S. Biomass Energy Alliance, America Soybean Association, Regional Council of Rural Counties, Women Involved in Farm Economics, Governors' Ethanol Coalition, Diagnostic Instrumentation and Analysis Laboratory, and the National Ethanol Vehicle Coalition.

F. Ferraro, Merrick
XCO-9-29030-01
Advances in Bioconversion of Biomass to Ethanol - Analysis of Single Stage Dilute Acid Hydrolysis
6/18/99 - 6/17/01
This subcontract was originally placed in order to advance alternative conversion technologies that had not previously been considered. Merrick/HFTA submitted a final report under this subcontract during this period. The subcontract was initiated as a single-stage dilute nitric acid process, but due to unsatisfactory results a two-stage approach was pursued. The first stage hydrolysis conditions using 0.10 - 0.15 wt % for 6 to 8 minutes at 175°C produced up to 90% pentose sugar yields. Eleven percent of the glucose sugars were also recovered. In the second stage, 35 to 45% of the glucan in the feed was converted to glucose. As per the analysis of ProForma Systems Inc., a 14.6 MM gallon per year facility assuming 55 gallons per ton of feedstock would require a capital investment of $37.8 MM with a gross profit of 30% when selling ethanol at $1.20 per gallon. The HFTA process is one that could potentially be considered by the state of California for development. Pretreatment specialists at NREL reviewed the technology, and various recommendations were suggested as a next step for process verification and a potential proposal to California funding agencies.

Lignin Combustion

Milestone Progress/Completion

P Milestone #285 – "Develop a Methodology to Predict Fuel Performance of Ethanol Production Residues" (9/30/01). It has been difficult acquiring diverse samples of residues for testing, however, we are working closely with industry representatives to arrange delivery of residue materials as soon as they are available. A methodology has been developed using the samples that have been obtained to date. The residues are

evaluated for fuel performance, including heating value and emissions. The methodology will continue to be refined as the sample base grows.

Progress Highlights and Issues

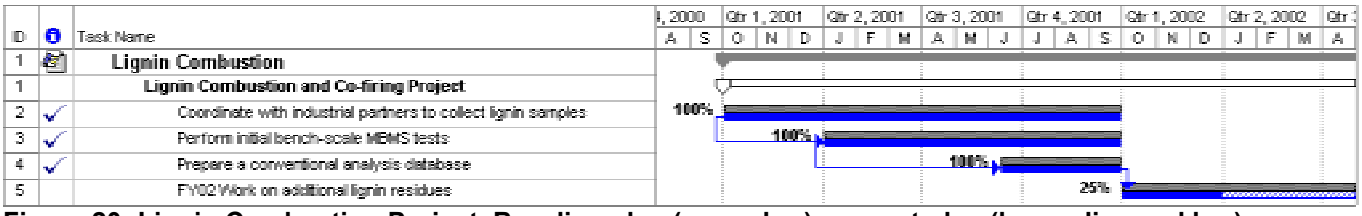

Figure 20: Lignin Combustion Project. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01.

Three new samples have been obtained and are undergoing testing. These samples represent two types of feedstocks and two different process approaches. Three softwood residues from three experiments are now included in the database. This is still well below the variability necessary to develop a predictive model. However, a preliminary comparison will be made as to the fuel performance characteristics of residues from different industrial processes (once testing is complete). Negotiations have also been underway to obtain a wider variety of residues. We have been promised residues from corn stover and MSW and will continue to seek other opportunities.

Cargill-Dow CRADA (CRD-00-092)

Milestone Progress/Completion

The first year of the Cargill-Dow CRADA has been completed. All five milestones in that statement of work were successfully completed on schedule. Research results and milestone reports are CRADA protected information.

Progress Highlights and Issues

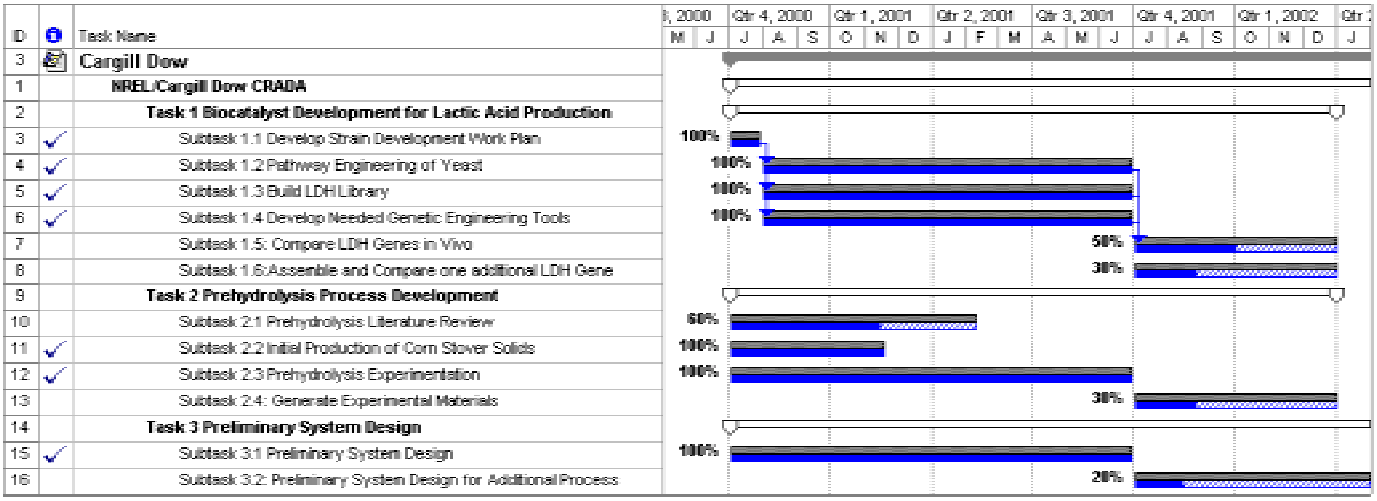

Figure 21: Cargill Dow CRADA. Baseline plan (upper bar), current plan (lower diagonal bar) and progress (lower solid bar). Status date 9/28/01.

This CRADA project is part of a one-year award by DOE to Cargill Dow LLC to help the company prepare to use cellulosic material such as corn fiber of corn stover as feedstock instead of starch for its new biobased polylactic acid (PLA) plastic. Cargill Dow is already producing and marketing PLA under the trade name NatureWorks™. Success will allow Cargill Dow to use lower cost feedstock, thereby allowing expansion of the market for PLA and perhaps make it possible to create ethanol or other chemical products in conjunction with PLA production.

Additional funding from July-December 2001 has been received and the work has been defined at an initial level. A final statement of work with milestones is in progress.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

- Rafael Nieves presented at the RBEP-sponsored Ethanol Workshops in Alaska, Minnesota, Nebraska, Pennsylvania, and Puerto Rico.

- On May 24, 2001, Rafael Nieves attended the Conversion Technologies for Municipal Residuals Forum in Sacramento, California. The event was sponsored by the California Integrated Waste Management Board.

- On May 7, 2001 Rafael Nieves was invited to present at the Western Regional Symposium for the Solid Waste Association of North America (SWANA) in San Luis Obispo, California.

- Rafael Nieves presented at the second meeting of the Louisiana Biomass Council in Baton Rouge, Louisiana, on August 7, 2001.

PROCESS ENGINEERING AND ANALYSIS

Summary of Technical Achievements or Results

The Process Engineering and Analysis team completed several important studies in this reporting period. These studies investigated cross-cutting areas not covered by the research based projects, such as improving the stover to ethanol process design, carbon and energy balance modeling and sugar separation technology. The process engineering team strives to understand and obtain the information that is needed for scaling-up the processes under study. Activities in this reporting period include:

- Publishing a process design report for corn stover to ethanol.
- Developing out-year scenarios to understand the effect of current and potential research efforts on bioethanol economics.
- Developing a current technology process design that documents the state of biomass to ethanol technology at NREL.
- Completing a carbon balance and energy balance for the stover to ethanol process.
- Developing and assessing USDA/NREL project phase II planning for a co-location scenario.

In addition, the Process Engineering team supported the research efforts of almost all of the Biofuels research projects; details are listed in the respective project sections.

- Performed an economic comparison of batch vs. flow through pretreatment reactors from information collected with the Harris Group.
- Developed a process design for the NEP project that provides research priorities and possible scenarios that, when coupled with research successes, create an economically compelling process design for stover-derived ethanol.
- Reviewed and modeled ammonia pretreatment processes with the respective members of the Biomass Refining CAFI.
- Completed a process design package for 2-stage dilute acid technology. Publication pending.
- Completed an economic evaluation and sensitivity analysis for the “superstover” pretreatment method.
- Determined and reported on how microbial strain characteristics translate to cost improvements in the ethanol process.

- Completed a pre-feasibility study on wheat straw for Washington State University.

- An 8% span of glucan plus xylan content in stover samples was shown to translate into a range of minimum ethanol selling price of 20 cents/gallon of ethanol. Differences in pretreatment yield among four of the stovers selected for this work was shown, at least in one case, to translate into a significant cost savings (~10 cents/gallon of ethanol) to the process.

- Performing preliminary economic analysis on distillers grains conversion to ethanol.

- Supporting the Gate 2 review of the lignin project with sensitivity analyses and by finding reviewers and valuable participants.

General Technical or Scientific Progress

Milestone Progress/Completion

C milestone # 237 – "Economic Evaluation of Various Pretreatment Technologies" (6/30/01, extended to 9/30/01). This milestone was completed with an overview analysis of the CAFI-represented technologies and an economic evaluation of several. From the CAFI's future work under the USDA project, data on one feedstock (stover) for several processes will provide the opportunity to perform additional analyses.

C Milestone # 239 - "Joint Publication with USDA on Process Economics Phase 2 Project," was extended to 8/31/02 due to USDA and NREL program priorities. Co-location scenarios for starch and cellulosic feedstocks were developed and approved by both institutions and block flow diagrams of the scenarios were presented at the Minneapolis Fuel Ethanol Producer's Workshop. An updated simplified NREL model (with purchased enzymes) has been developed. This is an important first step in the work, because there have been significant changes to the process design since Phase I was initiated. This simplified model has proven useful outside of this project, for distribution to universities and other research institutions that can make use of the Aspen model to better direct their efforts.

Progress Highlights and Issues

The corn stover process design report titled "Process Design Update" was published. This is the second design report the Biofuels program at NREL has created; the first was in 1999 for poplar feedstock. These reports, which are the culmination of process design studies with engineering firms, NREL research and subcontract research, make information on the conceptual design, operating and capital costs readily available to the ethanol community.

An outyear analysis was performed to show the effect of research projects, both near and long term, on the process economics of corn stover to ethanol. Stage gate project goals on both the blue (commercialization) and orange (exploratory) lines were translated into process parameters and their effect on the production cost analyzed.

Delta-T's contract "Evaluation of the Potential for the Production of Lignocellulosic Based Ethanol at Existing Corn Ethanol Facilities" was completed in this period. The last of six Bridge subcontracts, this study looked at gasification and catalytic syngas conversion to produce ethanol from stover. Delta-T also evaluated a dilute acid (Brelsford) process to convert distiller's grains to ethanol and animal feed at the Chippewa Valley Ethanol dry mill in Benson, Minnesota. They determined that while distillers grains conversion was feasible, the payback period was greatly affected if the existing site did not have excess fermentation capacity. The study also suggested further investigation of gasification and syngas conversion as an advanced (not near-term) technology.

A model, coined the "NREL Current State of the Technology," was developed to document where bioethanol technology is at NREL. Combined with research targets, this model helps to determine where the gaps in understanding are so that program efforts can be directed at closing them.

Energy and carbon balances were completed for the corn stover design case. These balances show where the energy or carbon is entering and exiting the process. In both cases, the primary entrance was in the feedstock. This analysis shows the process efficiencies of carbon and energy conversion to ethanol and byproducts, such as electricity. Further work will be done to interpret the results and compare them to other processes, such as corn dry mills and biomass power generation.

Subcontractors

J. Sinor, Jerry Sinor Consulting, Inc.
TXE-0-29113-01
Market Analysis for Lignin Products
1/12/00 - 1/11/02
A report reviewing the feasibility of producing jet fuels from lignin is to be published by the end of FY02. Mr. Sinor also worked to determine EPA contacts that would be good sources of information and feedback on the lignin fuel project.

A. Van Draanen, Ingram-Howell
ACL-1-30123-01
Hydrolysis and Fermentation of Pulp and Paper Wastes
10/9/00 – 11/23/01
Task 4 (pilot-scale hydrolysis) is yet to be completed.

A. Van Draanen, Ingram-Howell
ADZ-1-31088-01
Permitting of Biomass Hydrolysis and Fermentation Facility
7/3/01 – 11/2/01
Subcontractor shall identify the permitting issues and develop permit applications associated with a Biomass Hydrolysis and Fermentation Facility in Washington State. There are six tasks: project work plan, regulatory review, air permit development, environmental permit development, other permits and waivers, and final report.

Ron McNulty, Delta-T Corp
ZCL-0-30008-01
Evaluation of the Potential for the Production of Lignocellulosic Based Ethanol at Existing Corn Ethanol Facilities
3/02/00 – 9/30/01
Completed review (with final report) of distillers grains conversion via acid-catalyzed pretreatment and enzymatic hydrolysis (near term technology) and gasification with syngas fermentation (longer term).

M. Ladisch, Purdue/Williams
ZCO-1-31023-01
Bridge to Corn Ethanol - Phase 2 Project
2/22/01 – 2/28/02
An experimental plan was developed by Purdue/Williams to address the key technical issues of conversion to corn fiber to ethanol through demonstration at the Williams facility. Due to staffing availability, the project has slipped about one month and the contract period of performance has been extended from 11/01.

L. Montague, Harris Group
ACO-1-30131-01
Lignin Process Design Confirmation and Capital Cost Evaluation
3/30/01 – 11/30/01
Process design review was completed and capital costing is underway.

Gregg Morris, Pacific Institute for Studies in Development, Environment and Security
XDH-0-30010-04
Logistics Support and Development of a Bioethanol Market in the Western US – Bioethanol/Biomass Power Co-location Study
8/15/00 – 12/31/01
This study is analyzing the economic feasibility of co-locating cellulose to ethanol production facilities with existing biomass power plants in California. It has been extended to include an investigation of co-locating such facilities with coal fired plants in the U.S., as such a combination has promise to provide many of the same benefits as co-location with a biomass power plant. Biomass power co-location scenarios were completed in this period and the final data for coal co-location are being analyzed.

Neoterics International
LCO-1-31055-01
Process Engineering Support for Biotechnology Center for Fuels and Chemicals
7/5/01 – 1/5/01 (with 6 month option)
Working on the CAFI pretreatment modeling and economic evaluation. Two ammonia pretreatments modeled during this period.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

- Ibsen, K. Presentation at the Mars, Pennsylvania Ethanol workshop titled "Ethanol for Rural America," August 2001.

- Ibsen, K. Meeting at TVA to discuss potential collaborations. August 2001.

- Jechura, J. Attend the Green Design Initiative Carnegie Mellon Ethanol Workshop, July 2001.

- Ibsen, K. Meeting at USDA-ARS ERRC to discuss potential collaborations, July 2001.

- Aden, A. Visit to National By-products for Biodiesel project. June 2001.

- Ibsen, K. Presentation at the North Carolina Ethanol Workshop and visit to Anco Eaglin. June 2001.

- Ibsen, K. Attend Distillers Grains Council Symposium in Louisville, KY, May 2001.

- Jechura, J. Attend the Utah Ethanol workshop titled "Biofuels in Utah's future: A dialog," May 2001.

Scientific Meetings: Papers/Posters Presented or Recently Accepted for Presentation

- USDA Phase I joint report "Determining the Cost of Producing Ethanol From Corn Starch and Lignocellulosic Feedstocks" was co-presented at the Fuel Ethanol Workshop in June 2001 by A. McAloon (USDA-ARS) and K. Ibsen.

- "Life Cycle Energy and Environmental Impacts of Bioethanol Made from Corn Stover" poster was presented at the 23rd Symposium for Fuels and Chemicals, Breckenridge, CO, May 2001 by A. Aden.

- "Improved Corn Stover to Ethanol Process Design and Economics" poster was presented at the 23rd Symposium for Fuels and Chemicals, Breckenridge, CO, May 2001 by M. Ruth.
- "Process Design and Economic Analysis for Production of Electricity, Fuels, and Chemicals" paper was accepted for presentation at the Fifth Annual Biomass Conference of the Americas, Orlando, FL, December 2001 by M. Ruth."Expanding the Biomass Sugar Platform: An Investigation of Sugar Separation & Purification Techniques"poster was accepted for presentation at the Fifth Annual Biomass Conference of the Americas, Orlando, FL, December 2001 by J. Jechura.

RENEWABLE DIESEL

Summary of Technical Achievements or Results

A recent project conducted by Brookhaven National Laboratory on the behalf of NREL showed that using B20 in home heating boilers could reduce NO_x emissions and does not require any modifications to the systems. Emission and performance tests on an in-house commercial boiler system should be available by the end of the fiscal year. Brookhaven and NYSERDA will be conducting a commercial test in an industrial boiler starting this fall. An ongoing field test with Warwick School District in Rhode Island using three different blends (10%, 15%, and 20%) of biodiesel with home heating oil has been running in three elementary school buildings with no problems since February. A consortium of industry oil heat organizations is interested in conducting a final set of research tests this fall and winter before commercializing the fuel.

Planning meetings for the Renewable Diesel Workshops were held in Sacramento, Seattle, Cedar Rapids, and Albany. The Sacramento and Seattle meetings were held on September 25th and September 27th, respectively with over 100 attendees at each one. The remaining workshops will be held during FY02. The support of the Energy Offices and other cosponsors has been invaluable. BBI is providing technical support under a subcontract.

Robert McCormick and Richard Parish (for DOE) prepared a review of E-diesel technical and infrastructure issues. A workshop with industry is being planned to determine what the E-diesel research agenda should be for industry, DOE, and others. A working meeting concerning these and other E-diesel issues will be held in Chicago in October.

The Puerto Rico biodiesel project is progressing well. The University of Puerto Rico has come up with a novel processing technology for esterification and the City of Caguas started their biodiesel demonstration in the fall. The project has received a lot of local press and the support of local political organizations. Phase II funding will expand the project and move it towards industrial development.

Two solicitations were made for the program: (1) RFP for developing course work for four biodiesel college level courses and (2) RFP for conducting a demonstration of trap grease conversion to biodiesel and preparing a feasibility study for the same. The response has been very encouraging and negotiations are underway with selected respondents.

Several final reports were received from contractors wrapping up efforts in several key areas, including the multifeedstock project with IGT, UC Davis, and CSM. Pacific Biodiesel's project with trap grease analysis will be descoped and closed out. Oxidative stability reports will be reviewed and released to ASTM and the industry for peer review.

Nohemi Zerbi, Anthony Turhollow, and K. Shaine Tyson reviewed the University of Idaho mustard project in July 2001. Several subsequent discussions with the PI's and

university management have been held to improve communication products. Contract modifications are under negotiation for year three of this activity.

General Technical or Scientific Progress

Milestone Progress/Completion

C Milestone # 335 – "Renewable Diesel Workshops" (9/30/01, remaining workshops will be rescheduled as a C Milestone in 02). Two of the five renewable diesel workshops were held in September with over 100 attendees at each. Two other workshops have been scheduled. A contract was placed with BBI for logistical support. The milestone dates were rescheduled for Cedar Rapids, Albany, New York and the southeast.

P Milestone #338 –"Renewable Diesel Evaluation" (9/30/01, rescheduled as a C Milestone in 02). A review of technical and infrastructure issues associated with potential renewable diesel fuels has been drafted. A strategic plan is under development in cooperation with the Center for Transportation Technology Systems at NREL which also conducts R&D on renewable diesel fuel engines and technologies.

P Milestone #337 – "Industrial Partners for Mustard" (9/30/01). The University of Idaho Pesticide Registration program is holding some discussions with several small pesticide firms in CA that may be early supporters of the project. Several partners for field testing the pesticide have been identified.

P Milestone #336 – "Reduce NO_x Emissions" (9/30/01). Project complete on 4/30/01. A final report was received on August 24, 2001. The report will be published in October 2001.

P Milestone #334 – "Trap Grease Consortium" (9/30/01). Several proposals were received and a contract is expected to be set in the first quarter of FY02 with the selected consortium.

Progress Highlights and Issues

Collaborative Activities

NREL has received an unsolicited proposal from the Massachusetts Oilheat Council and the New England Fuels Institute (and several other consortium participants) for an industry controlled project to examine the issues associated with using B20 in heating oil systems. This project would define industry acceptance and would permit biodiesel to be sold commercially in the following heating season. The proposal is requesting matching funds of $32,300 from NREL; MOC has already approved $32,300 of their own funds. NREL has asked the Division of Energy Resources of the Commonwealth of Massachusetts and the Northeast Regional Biomass Energy Program (RBEP) to split the $32,300 request.

A biodiesel heating oil blend demonstration started in March involving NREL, the Rhode Island Energy Office, Warwick School District, Global Companies, Brennan Oil, and World Energy. Blends of 10%, 15%, and 20% and a heating oil control are being used at

four public school buildings for a year. The project began in March and is being managed under the petroleum infrastructure project with Advanced Fuels Solutions.

The Life Cycle Analysis of Yellow Grease Biodiesel Production with the Fats and Protein Research Foundation (FPRF) is underway. An industry survey has been drafted with input from both organizations and submitted to industry. The survey should be released by industry in the fall. The life cycle analysis on grease and fat biodiesel will be finalized in FY02.

A final report from U.C. Davis describing the results of toxicology and mutagenicity tests on multifeedstocks has been received. No significant differences were detected between the various biodiesel fuels.

Cross-Program Research Activities

Cooperative efforts with the NREL Fuels Utilization Program include technical support for renewable diesel projects managed by G. Sverdrup, W. Clark, K. Vertin, and M. Frailey in the Center for Transportation Technology and Systems. A joint task with the Colorado School of Mines on Fischer Tropsch (FT) and Biodiesel blends has been completed under subcontract XCO-0-30088-01. A joint DOE-EPA test of biodiesel using a "sniffer truck" was postponed. K. S. Tyson is providing technical support.

NREL worked with OFD to submit a SRIR idea to the process. Other support activities included developing a strategic overview of the project for new DOE management.

Regional Biomass Energy Program Collaboration

NREL continues to provide technical support to the RBEP on a biodiesel project in NV and CA with Biodiesel Development Corporation (BDC), and to assist with program planning. Tyson also provides support for a new project with the National Biodiesel Board (NBB) and WRBEP. K. S. Tyson attended the spring meeting in Nebraska City, Nebraska in April.

Technology Transfer/Industry Outreach

The biodiesel fact sheet DOE/GO-102000-1048 was updated and reprinted. A "Biodiesel Handling and Use Guidelines" report has been finalized with industry review and printed as NREL/TP-580-30004. Tyson provided input to a new Clean Cities Biodiesel Fact sheet and to several other DOE publications. A presentation to the Coordinating Research Council Emissions Subcommittee on biodiesel emissions and engine performance was well received by industry leaders. Five workshops are underway with state agency cosponsors to provide more information about renewable diesel fuels.

Subcontracts

Brookhaven National Laboratory
DAR-1-31053-01
"Heating Oil Assessment" subcontract will be completed in first quarter FY02.

University of Idaho
XCO-9-29095-01

"Mustard Crops for Biodiesel Phase II" made progress. A review meeting was held and some changes were discussed. Further modifications may be reflected in the third year of the contract.

Colorado School of Mines
XCO-0-30088-01
"NO_x Solutions for Biodiesel" has been completed as of 4/30/01. A final report has been received and will be printed in the fall of 2001.

Colorado School of Mines
ACG-8-17106-01
"Health Related Emissions From Biodiesel…" The remaining portion of this contract consists of basic combustion chemistry to determine how and where NO_x is formed. Contractor completed testing and a draft report was received in September.

Southwest Research Institute
AXE-9-29057-01
"Biodiesel Emissions from Locomotives" has been completed. A draft report has been peer reviewed by several industry organizations and a final report will be received by first quarter FY02.

Southwest Research Institute
ACG-7-17066-01
"Oxidative and Thermal Stability Testing Methods for Biodiesel" was extended through March FY02 to allow completion of the work. Most of the work has been accomplished at this time.

Pacific Biodiesel
ACO-9-29045-01
"Composition Analysis of Waste Grease." Pacific Biodiesel recently submitted 50% of the composition data. The literature review was resubmitted and accepted. The contract will be descoped and closed.

Advanced Fuel Solutions
XCO-0-30044-01
"Petroleum Infrastructure Development" has been modified to add $30,000 to support biodiesel education with the heating oil industry and support the Warwick project. A new task to examine additive issues in biodiesel blends was added.

Proforma Systems
TXL-9-29031-01
"Biodiesel Process Engineering and Economics" has progressed very slowly. A rough draft of soy and grease biodiesel systems at three scales is still not available.
University of Puerto Rico
ACO-1-30119-1
"Grease Biodiesel for Puerto Rico" OFD and ED have jointly funded a project with the University of Puerto Rico Mayaguez and Panzardi ERM. Phase I is off to a good start

with resource assessment, market evaluation, and technology/testing methods underway. A site visit with DOE sponsors was conducted in March to develop ideas for Phase II. A Phase II proposal was submitted to DOE for $200,000. Significant economic benefits for the region appear to be present. K. S. Tyson has held several discussions with producers interested in investigating opportunities in Puerto Rico. The contractor has identified demonstration sites and the City of Caguas has offered a $20,000 cost share. The Energy Administration Office has offered $25,000 cost share for the demonstrations.

Subcontracts to be Placed

Biodiesel Education Tools – NREL has received two proposals, and is negotiating with both firms at this time bring the best of both proposals together.
Mustard Pesticide Market Assessment – postponed until FY 02 due to contractor delays.
Trap Grease Production Consortium –NREL received two proposals and is negotiating with one firm at this time.
Animal Fats for Biodiesel Life Cycle Analysis – a SOW was developed and an RFP will be released in early FY02.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

- March 31-April 2, San Francisco, CA, National Association of Fleet Administrators (NAFA).

- April 3 – 5, Nebraska City, NE, Western Regional Biomass Energy Program Semi Annual Review Meeting.

- April 29 – May 2, Brookhaven National Laboratory, National Oilheat Conference, presentation and paper.

- May 8, Washington, DC, Presentation for the USDA Biobased Products Council.

- May 23, San Francisco Retail B100 pump dedication. Meeting with San Francisco Water Transit Authority and Blue and Gold Ferries.

- May 25, NREL, Presentation to Christian Rakos, Austrian Energy Center.

- June 6, CA, Renewable Diesel Workshop Planning Meeting, Sacramento, CA.

- June 21 – 22, St. Louis, MO, National Biodiesel Board Meeting.

- June 25, Seattle WA, WA Renewable Diesel Workshop Planning Meeting.

- June 26-28, Moscow Idaho, Univ. of Idaho Project Review.

- June 29, IA Renewable Diesel Workshop Planning Meeting, Cedar Rapids, IA.

- July 10, Albany, NY, NY Renewable Diesel Workshop Planning Meeting.

- August 15, Lake Tahoe, NV, CRC Emissions Subcommittee. Biodiesel presentation.

- September 11, Fort Collins, CO, IR-4 Pesticide Registration for Food Crops Meeting.

- September 25, Sacramento, CA, Renewable Diesel Workshop.

- September 27, Seattle, WA, Renewable Diesel Workshop.

Scientific Meetings: Papers/Posters Presented Or Recently Accepted For Presentation

- Brookhaven National Laboratory, National Oilheat Conference, presentation and paper. April 29-May 2.

- Large Booth display was developed for NAFA meeting.

- Presentation for 5th Biomass of the Americas was accepted, however meeting was cancelled.

Scientific Journals: Papers Accepted for Publication

- Tyson, K. S. and Nazzaro, P. "Biodiesel as a Heating Oil Blend Stock." In *Proceedings of the 2001 National Oil Heat Research Alliance Technology Conference*, pg. 139-152. BNL-52625.

NREL Reports

- Tyson, K. S. *Biodiesel: Handling and Use Guidelines*, March 2001. NREL/TP-580-30004.

- Tyson, K.S. *Biodiesel—Clean, Green Diesel Fuel,* September 2001. NREL/GO-102001-1449. Updated.

COMMUNICATIONS

Summary of Technical Achievements or Results

During the second half of FY 2001, a number of new projects were completed to help communicate with the Biofuels Program's identified key external stakeholder audiences. These audiences include the ethanol industry, the agricultural community, environmental interests, state government, the forestry industry, bioenergy/bioproducts interest, and the transportation/end use sector. Communications has completed the following projects:

Newsletters

- Biofuels News Summer 01 – National Energy Policy and Biofuels/ Proposed Budget for Bioenergy

Fact Sheets/Brochures

- Biofuels for Your State
- Bioethanol — Moving into the Marketplace (revision)
- Biodiesel — Clean, Green Diesel Fuel (revision)

Web Sites

- EREN/NCSL State Energy Alternatives Site Biofuels Pages
- Biofuels Program Site Redesign
- Routine updates to Biofuels Program, Biofuels Information Center, and OFD Web pages and document databases

Trade Show Exhibits

- Exhibited for Biodiesel at National Association of Fleet Administrators' Fleet Management Institute in San Francisco in April
- Exhibited for Biofuels Program at Fuel Ethanol Workshop in St. Paul in June
- Exhibited for Biofuels Program at National Conference of State Legislatures annual meeting in San Antonio in August
- Prepared to exhibited for Biofuels Program and DOE Bioethanol Pilot Plant at postponed Biomass Conference of the Americas in Orlando in September

General Progress

Milestone Progress/Completion

C Milestone #236 - "Update Biofuels Web Site and Implement a System to Insure That All Remain Updated" (4/31/01). The National Biofuels Program Web site was created in FY 1999 and has been expanded and improved since. This milestone was completed on schedule. In addition, during the second half of FY 2001, we completed a major redesign of the Web site home page and high-level pages. Based on experience, survey of selected users, and Web support personnel, the site was redesigned to improve navigation and user utility, to match better with concurrent Office of Transportation Technology Web page redesign, and to add new features. Work to fill in content for some of the new pages and update and improve existing pages will continue in FY 2002. Maintenance and improvement of the Biofuels Program Web site is a continuous activity.

P Milestone #290 - "Produce a Biofuels Section for the EREN State Energy Alternatives Web Site", (4/30/01). This milestone has was rescheduled for 6/30/01 and largely completed by August 2001. Addition of state-specific information and additional maps will continue during FY 2002. This was an important opportunity to get good information about biofuels to a key stakeholder audience—state level decision makers belonging to the National Conference of State Legislatures. The new pages also provide a source of information about current ethanol production and use. This is only minimally covered on the Biofuels Program site because it is not a major focus of the Biofuels Program, but it is of interest to many of our stakeholders.

Progress Highlights and Issues

In planning and carrying out communications activities for FY 2001, we have instituted a more concerted and effective planning, tracking, and production process. We have developed a cohesive communications team at NREL that works well with Biofuels Program personnel, have made communications a more integral part of the Biofuels Program, reestablished timely production of Biofuels News, responded to a rapidly increasing volume of telephone, email, and Web inquiries in a timely and helpful manner, and improved relations with stakeholder organizations. We made substantial progress toward completing an ambitious slate of planned publications and other projects, but have several projects remaining for completion in FY 2002. To finish those projects and additional identified priorities, we intend to allocate additional personnel resources to biofuels communication in FY 2002. While continuing to produce new publications, Web pages, and other materials, we also intend to place greater emphasis during FY 2002 on dissemination of information we have already produced.

During the second half of FY 2001, we issued a contract with a public relations firm to provide the Biofuels Program with advice on reaching the agricultural community. This effort was motivated in part by the Program's emphasis on corn stover as a potential feedstock and will enhance efforts to determine whether growers would be amenable to harvesting stover for ethanol production and to start soliciting interest in doing so. The contract included assessment of baseline knowledge and attitudes, identification of key audiences and messages, evaluation of existing communication products, and planning for effective new communication efforts. The final report—to be received at the close of FY 2001 or early in FY 2002—should provide key guidance for Biofuels communication efforts in the coming years. The recommendations—which will likely have considerable applicability to reaching other stakeholders as well as agricultural ones—are expected to call for a more aggressive approach to working with the media, interest groups, and others to let the world know about what the Biofuels Program is doing and is all about.